AD_____

Award Number: W81XWH-04-1-0104

TITLE: The Effect of Hypotensive Resuscitation and Fluid Type on Mortality, Bleeding, Coagulation and Dysfunctional Inflammation in a Swine Grade V Liver Injury Model

PRINCIPAL INVESTIGATOR: Martin A. Schreiber, M.D.

CONTRACTING ORGANIZATION: Oregon Health & Science University
Portland, Oregon 97239

REPORT DATE: January 2012

TYPE OF REPORT: Addendum to Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
January 2012	Addendum to Final	8 December 2003 – 7 December 2011

4. TITLE AND SUBTITLE

The Effect of Hypotensive Resuscitation and Fluid Type on Mortality, Bleeding, Coagulation and Dysfunctional Inflammation in a Swine Grade V Liver Injury Model

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-04-1-0104

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Martin A. Schreiber
Jerome Differding

E-Mail: differdi@ohsu.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Oregon Health & Science University
Portland, Oregon 97239

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Purpose: To determine the efficacy of resuscitative fluids currently utilized in military and civilian settings on the physiologic response to uncontrolled hemorrhage. **Scope:** Swine were subjected to a couple of different models over the length of this multi-year trial. Animals either underwent just a Grade V liver injury or were subjected to the lethal triad of trauma which included hemorrhage, hypothermia and coagulopathy. Over the course of this trial animals were randomized to several resuscitation models including but not limited to normal saline (NS), Lactated Ringer's (LR), 7.5% saline with 3% Dextran (HTS),Hextend (HEX),Lyohilized Plasma (LP), Fresh Frozen Plasma (FFP), and Packed Red Blood Cells(prbc's). Physiologic parameters, coagulation assays and inflammatory mediators were compared. **Major Findings:** Significant findings will be highlighted throughout the body of this report but of most note, LR performed better than NS and LP performed as well if not better than FFP.

15. SUBJECT TERMS
Uncontrolled hemorrhage, resuscitation, animal model, coagulopathy, swine, lyophilized plasma

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	89	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

The Effect of Hypotensive Resuscitation and Fluid Type on Mortality, Bleeding, Coagulation and Dysfunctional Inflammation in a Swine Grade V Liver Injury Model

Introduction / Statement of Work

The leading cause of battlefield deaths during the current conflict is hemorrhage. Based on autopsy data, 17% of patient who have died could have been saved if hemorrhage was controlled and resuscitation was adequate. Only 31% of patients with exsanguinating hemorrhage can be controlled by means presently available to the first responder and 69% of all hemorrhagic deaths on the battlefield are from injuries not well controlled by medics. Adequate care of these patients at the Combat Support Hospital requires improved noncompressible hemorrhage control and resuscitation to restore normal coagulation and metabolic processes. This problem is magnified by the fact that, similar to civilian casualties, severely injured military casualties are coagulopathic upon arrival to the CSH. The solutions currently used for resuscitation include crystalloid, colloid and blood products that are devoid of coagulation factors. This type of resuscitation worsens the initial coagulopathy of trauma and increase the tendency for more bleeding. In the presence of tissue damage and hypoperfusion due to hemorrhage, the immune system and the coagulation pathways are activated. The delicate interplay between these systems can fail, leading to increased bleeding. Thus, in the presence of uncontrolled bleeding there must be a tight coupling between coagulation, immune function and metabolic resuscitation to effectively treat the victim of severe trauma. This research effort will address this complex interplay and will define and evaluate the combination of currently available and future products to treat patients with uncontrolled bleeding and metabolic insufficiencies.

The overall goal of this statement of work is to complete research that will determine the optimal resuscitation strategy in a severe swine model of uncontrolled hemorrhage. It will determine which of the fluids currently available are most effective at correcting coagulopathy and it will work to develop new resuscitation strategies that can be available on the battlefield. In addition, we have noted that inhalational anesthetics have a profound effect in our model in that they decrease systemic vascular resistance and cause cardiac suppression. We hypothesize that a total intravenous anesthesia regimen (TIVA) will reduce these affects and produce a more favorable hemodynamic profile. Specific Aim 7 describes a comparison between our standard inhalational anesthetic and TIVA.

The model described in Specific Aim 3 is designed to recreate the battlefield scenario. It combines a long bone fracture, with severe soft tissue injury and the previously described grade V liver injury. It is similar to a blast injury. This model is also designed to recreate the lethal triad of acidosis, coagulopathy and hypothermia.

The fluids described in Specific Aim 8 have been utilized for resuscitation of soldiers in Operation Iraqi Freedom. Lactated Ringer's is a standard isotonic fluid commonly used for resuscitation. 5% hypertonic saline has several hypothetical advantages. It results in increased intravascular expansion with smaller fluid volumes that are easier to transport in austere settings and it has been shown to reduce inflammation and decrease multiple organ failure. Hextend is hetastarch in a balanced salt solution that also results in increased intrasvascular expansion with smaller volumes. In addition, resuscitation with Hextend has been shown to result in decreased

blood loss. Fresh frozen plasma has excellent volume expansion qualities and it contains coagulation factors. Its effects as a sole resuscitation fluid have not been well-studied. The effects of these fluids on hemodynamics, coagulopathy and inflammation will be compared.

Specific Aim 1. To determine the arterial pressure at which re-bleeding occurs following a grade V liver injury

Determine the systolic arterial blood pressure at which re-bleeding occurs following a grade V liver injury in swine. A grade V liver injury will be induced. Following a 1 hour period of permissive hypotension, resuscitation with lactated Ringers at 100 ml/minute will be initiated and blood pressure will be monitored continuously. The abdomen will be left open and the liver injury will be examined for evidence of re-bleeding. The venous and arterial pressures at which re-bleeding occur will be recorded and averaged for a maximum of 20 consecutive animals. (Months 1-3, 20 Animals)

Specific Aim 2. To define the optimal endpoint of resuscitation and the optimal resuscitation fluid in an uncontrolled hemorrhagic shock model after prolonged permissive hypotension

a. Modify a well-described grade V liver injury model in swine to replicate the battlefield scenario. Modifications will include room air conditions, the use of saffan as the anesthetic agent, use of room temperature intravenous fluids and no attempt will be made to keep the animals warm beyond blankets. (Months 4-6, 20 Animals)

b. Determine baseline cytokine values in the animal population and cytokine alterations based on the surgical preparation. Animals will undergo anesthesia and immediate euthanasia. Cytokine measurements will be performed on lung, kidney and liver tissue. A second group of animals will undergo placement of invasive lines, celiotomy, splenectomy and a total of 24 hours of anesthesia. The animals will then undergo euthanasia and cytokine measurements. (Month 7, 12 Animals)

c. Determine the optimal resuscitation endpoint and resuscitation fluid. The model described in specific aim 1 will be used. Animals will undergo injury followed by a 1 hour period of uncontrolled hemorrhage. They will then be randomized to 4 resuscitation endpoints and 4 resuscitation fluids for a 24 period. The endpoints of resuscitation will be: 1) Full resuscitation - these animals will be resuscitated to their baseline blood pressure. 2) Mild hypotensive resuscitation - these animals will be resuscitated to 10 mmHg below the re-bleeding blood pressure defined in specific aim 1. 3) Severe hypotensive resuscitation - these animals will be resuscitated to 30 mmHg below the re-bleeding blood pressure and 4) No resuscitation - these animals will serve as controls. The resuscitation fluids will be: lactated Ringers, 5% hypertonic saline, Hextend and PolyHeme. Primary outcome variables will be mortality, time to death, blood loss, extent of coagulapathy, resuscitation requirements and acidosis. (Months 8-56, 143 Animals)

d. Determine the effect of the different resuscitation regimens on dysfunctional inflammation. Lung, kidney and liver tissue will be processed for mRNA and protein extraction. Interleukin-6 (IL-6), IL8, Granulocyte Colony Stimulating Factor and Tumor Necrosis Factor - alpha mRNA

will be measured by quantitative reverse transcriptase polymerase chain reaction. Nuclear factor-kappaB and signal transducer and activator of transcription (STAT)3 activity will be measured by electron mobility shift assay. (Months 7-60)

Specific Aim 3. Development of a Relevant Military Model of Combined Injury and the Lethal Triad

Animals will undergo invasive line placement and general anesthesia with isoflurane. They will then undergo laparotomy and splenectomy. Animals will be cooled to 33C using cool intra-abdominal fluid. A captive bolt gun will be used to create a soft tissue injury and a comminuted femur fracture of the right leg. Controlled hemorrhage will be performed removing approximately 24 cc/kg of blood. A grade 5 liver injury will be created and the liver will be packed with gauze sponges 30 seconds after injury. Resuscitation with 2 units of whole blood will be performed followed by resuscitation and maintenance of baseline blood pressure with lactated Ringer's solution. Laboratory studies will include coagulation assays to include the Thrombelastogram, arterial blood gasses and lactate. The model will be modified for survivability and to insure that hypothermia, coagulopathy and acidosis have been induced before proceeding to Specific Aim 4. (Months 26 – 28, 20 Animals)

Researchers at Oregon Health & Science University will be responsible for developing and standardizing this model. After model development is complete, the model will be exported to Harvard University and USAISR. Specific Aims 4 and 5 will be performed simultaneously at all 3 institutions to expedite the production of data and the speed with which these finding can be translated to the battlefield. This "subcontract" with Harvard and USAISR will cost $0.

Specific Aim 4. Optimal Hemostatic Resuscitation

Using the model described in Specific Aim 3, hemostatic resuscitation will be studied. Resuscitation with whole blood will be compared to component therapy. The efficacy of recombinant factor VIIa will also be studied. The primary endpoint will be post-treatment blood loss. Secondary endpoints will include survival and survival time, pre-treatment blood loss, laboratory coagulation variables and fluid requirements to maintain baseline MAP. IL-6, IL-8 and TNF will be measured in the serum and lung expression of these cytokines will also be measured. These studies will be performed simultaneously at OHSU, USAISR and Harvard in order to expedite the production of results. The work performed at USAISR and Harvard will be funded from independent funding not related to this grant. (Months 29 – 42, 80 Animals at OHSU alone)

Specific Aim 5. Lyophilized Plasma

These studies will be designed to develop and test the efficacy of lyophilized plasma. Using the model described in Specific Aim 3, different formulations of lyophilized plasma will be tested. When an acceptable formulation has been identified animals will be divided into 6 groups: Group 1 will receive lyophilized plasma alone, Group 2 will receive fresh frozen plasma

prepared from donor pigs, Group 3 will receive lyophilized plasma and red blood cells in a ratio of 1:4, Group 4 will receive fresh frozen plasma and red blood cells in a ratio of 1:4, Group 5 will receive lyophilized plasma and red blood cells in a ratio of 1:1 and Group 6 will receive fresh frozen plasma and red blood cells in a ration of 1:1. The primary endpoint of this study will be coagulation assays at the end of the study; additional endpoints will include survival, blood pressure, laboratory assays and measurements of dysfunctional inflammation. (Months 43 – 55, 70 Animals)

Specific Aim 6. Advanced Resuscitation Technologies

These studies will provide further evidence as to which blood components are needed and in what quantities, as well as when this therapy should be delivered. Studies will expand to new variants of FVIIa, fibrinogen, platelets or platelet-like compounds, anti-fibrinolytics and hemoglobin substitutes, and determine whether labile clotting factors are required. Additional studies will also determine the means and procedures by which successful therapies can be moved forward of echelon II and III. (Months 59-70, 80 Animals)

Specific Aim 7. Comparison of Inhalational Anesthetic to TIVA.

Animals will be induced with an IM injection of Telazol and an aural intravenous catheter will be placed. Animals will then be randomized to receive either isoflurane 1-3% or TIVA. Invasive lines will be placed and a Grade V liver injury will be made. After 30 minutes of uncontrolled hemorrhage, animals will be resuscitated to a mean arterial pressure of 60mmHg with lactated Ringer's and maintained at that blood pressure for 4 hours after injury. The primary endpoint of the study will be volume of fluid required to achieve and maintain the goal pressure. Secondary endpoints will include blood loss, metabolic parameters, coagulation parameters and inflammatory parameters. We hypothesized that the TIVA regimen will result in lower fluid requirements, less acidosis and decreased dysfunctional inflammation compared to isoflurane. (Months 34 - 36, 38 Animals at OHSU alone)

Using the model described above for Specific Aim 3, we will further compare the use of ketamine and isoflurane 1-3% by adding midazolam and buprenorphine to both anesthetic groups. This would allow better comparisons between the two groups, as the only difference would be between ketamine and isoflurane. Furthermore, this model would more accurately duplicate anesthesia regimens that are administered to humans. We will also utilize 6 swine as controls which will be anesthetized and euthanized with tissue harvesting to determine baseline inflammatory values within the population. Additionally, we will have a total of 12 swine as shams, 6 for the ketamine group, and 6 for the isoflurane group. These animals will undergo identical anesthesia, and operative interventions as the study animals within their respective group, only the sham animals will not receive a liver injury. This allows us to evaluate the hemodynamic and inflammatory impact of the surgical setup and operative interventions performed. (Months 18-20, 38 Animals at OHSU alone)

Specific Aim 8. Comparison of current military resuscitation fluids.

Animals will undergo a grade V liver injury followed by 30 minutes of uncontrolled hemorrhage. They will then be randomized to receive: Lactated Ringers, 5% hypertonic saline, Hextend, or FFP. Primary outcome variables will be mortality, time to death, blood loss, extent of coagulapathy, and acidosis. The effects of these fluids on hemodynamics and oxygenation will be determined. Inflammatory parameters will be measured in lung tissues. The purpose of this aim is to determine which of the currently used fluids has the best overall resuscitation profile. (Months 72-84, 60 Animals)

Study Timeline

Year	Dates	Aims	Studies
1	12/08/03 – 12/07/04	1,2	Hypertonic
2	12/08/04 – 12/07/05	2,7	LR vs. NS, Ketamine
3	12/08/05 – 12/07/06	2,3	FFX
4	12/08/06 – 12/07/07	4,7	FFX, Tiva
5	12/08/07 – 12/07/08	5	FFX2
6	12/08/08 – 12/07/09	5,6	FFX3, Nitric Oxide
7	12/08/09 – 12/07/10	6,8	TXA, Fluids 2010
8	12/08/10 – 12/07/11	8	Fluids 2010

Body

Specific Aim 1. To determine the systolic arterial pressure at which rebleeding occurs after a grade V liver injury in swine.

Introduction

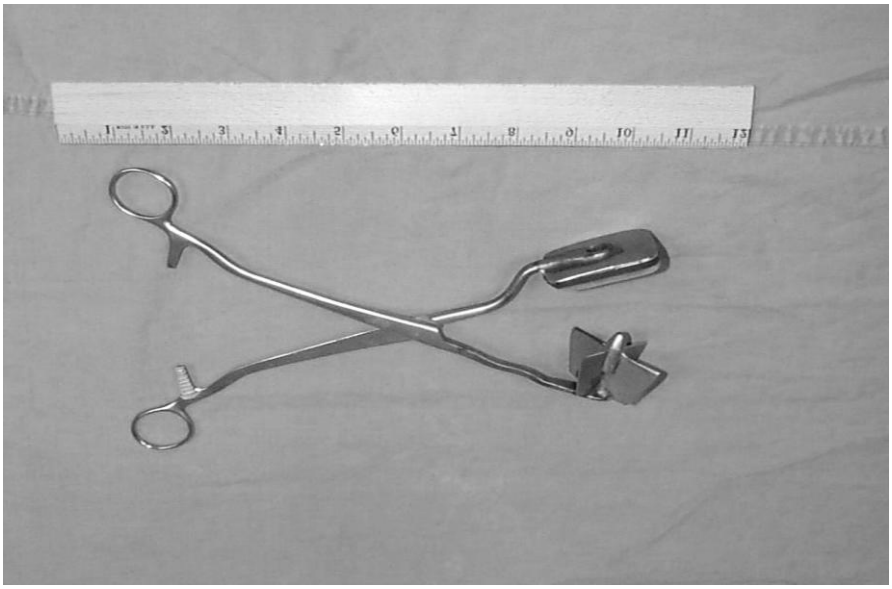
It is our hypothesis that resuscitation to a systolic blood pressure 10 mmHg below the rebleeding blood pressure after injury will result in increased survival and less dysfunctional inflammation than resuscitation to either baseline pre-injury blood pressure or resuscitation to 30 mmHg below the rebleeding blood pressure. We also hypothesize that the use of PolyHeme will result in improved outcomes following uncontrolled hemorrhage in swine.

Technical Objective

The specific objectives of this aim are: 1) to determine the systolic arterial pressure at which rebleeding occurs after a grade V liver injury in swine, 2) to determine the optimal endpoint of resuscitation and resuscitation fluid in an uncontrolled hemorrhagic shock model after prolonged permissive hypotension.

Model: Our laboratory has extensive experience with the liver injury model described in this application. The model involves the use of a liver clamp which creates an extensive stellate, crushing laceration simulating a combination of blunt and penetrating trauma. (See Fig. 1)

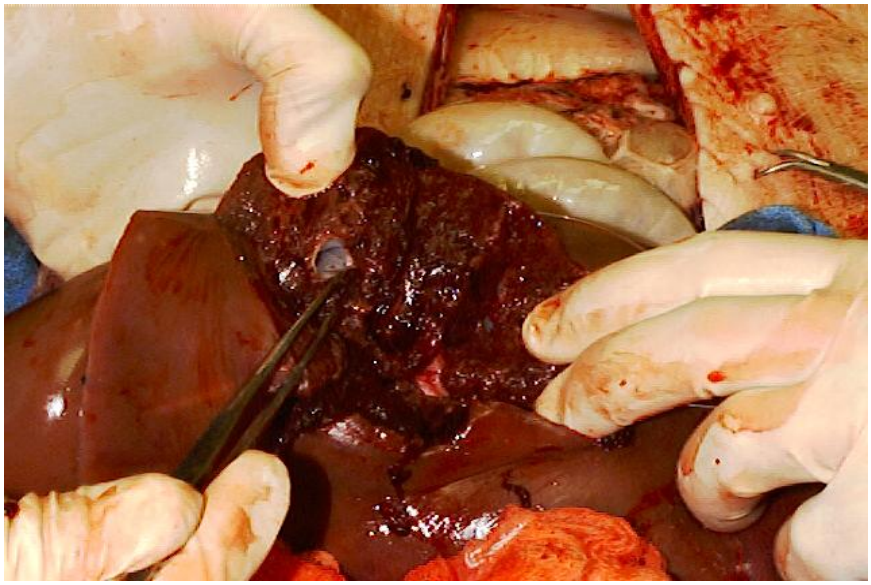
Figure 1. Clamp used for the pig liver injury model



The clamp is placed centrally in the liver and it produces laceration of 1-3 major hepatic veins consistent with a grade V injury as described by the American Association for the Surgery of Trauma Organ Injury Scaling of the liver.[76] The clamp also produces a significant amount of soft tissue injury. (See Fig. 2) This model has been used to test the efficacy of a dry fibrin sealant dressing as well as recombinant Factor VIIa in diminishing blood loss after injury. In untreated pigs resuscitated with lactated Ringers solution, the model results in a 50% mortality. The effects on blood loss, coagulation and inflammation are also well known.

Animal Plan: Female Yorkshire crossbred pigs of will be fasted for 16 hours the day before surgery. Water will be available ad libitum. On the day of the experiment, animals will be given pre-anesthetic medication consisting of Telazol^{R} 4mg/kg given intramuscularly. An aural 18 gauge intravenous catheter will be secured and flushed. A 7.0mm internal diameter cuffed endotracheal tube will be placed. An esophageal stethoscope, gastric tube and esophageal thermometer will be inserted. Animals will be placed in the supine position and the ventral cervical area and ventral abdomen will be clipped. An EKG monitor will be secured and continuous monitoring started. The endotracheal tube will be connected to the anesthesia machine with 1-3% isoflurane in 50% oxygen given for anesthetic maintenance. The tidal volume will be fixed at 10 ml/kg with a rate of 12 - 14 breaths per minute. Respiratory rate and tidal volume will be adjusted to maintain the PCO_2 close to 40 mm Hg. A left ventral cervical cut down will be made and 8F polyethylene catheters will be placed in the carotid artery and the external jugular vein. The arterial catheter will be used for continuous blood pressure analysis and blood sampling. Mean arterial pressure, systolic pressure, diastolic pressure and heart rate will be recorded and averaged every 10 seconds using a digital data collection system with a blood pressure analyzer. The venous catheter will be used for administration of study fluids. Additional venous lines will be placed in the suprahepatic and infrahepatic IVC to measure perihepatic venous pressures.

Figure 2. Example of liver injury.



At this point, isoflurane will be discontinued and an infusion of saffan at 13-16 mg/kg/hr will be initiated. Saffan is used in hemorrhage studies because it preserves the interaction between injury and cardiovascular reflex activity.[81]

A ventral midline incision will be made and a laparotomy will be performed. A splenectomy will be performed and the splenic hilum will be ligated with 0-silk suture. A splenectomy is performed in swine hemorrhage studies because the swine spleen is distensible and contains highly variable amounts of blood. This variable can affect bleeding in hemorrhage studies. The spleen will be weighed and warm lactated Ringer's solution will be infused at 3 times the weight to replace the removed volume of blood. A cystotomy will be made and a Foley catheter will be placed. The abdominal wall will be closed with towel clips.

The animals will be allowed to stabilize for a minimum of 15 minutes. The FiO_2 will be decreased to 21% and no effort beyond the use of blankets will be made to maintain the animals' temperature. These conditions replicate the combat scenario. After the stabilization period, the animals will undergo the Grade V liver injury. They will be allowed to bleed freely. After 1 hour, resuscitation with room temperature LR at 100 ml/min will be initiated. The liver injury will be observed for evidence of rebleeding. The arterial and venous pressures at which rebleeding occur will be recorded and averaged for 20 consecutive animals.

Specific Aim 2. To determine the optimal endpoint of resuscitation and resuscitation fluid in an uncontrolled hemorrhagic shock model after prolonged permissive hypotension

Introduction:

Exsanguination is the leading cause of death on the battlefield. Lifesaving interventions include arresting hemorrhage and initiating resuscitation. The ideal resuscitation of combat casualties has not been determined. The goal of this proposal is to determine the ideal resuscitation regimen of swine undergoing a Grade V liver injury followed by 30 minutes of uncontrolled

hemorrhagic shock. Fluids studied include lactated Ringer's (LR), Hextend and various concentrations of hypertonic saline. Fluids were evaluated based on their effects on mortality, metabolic changes, blood pressure, tissue oxygenation and inflammatory changes measured in the lung.

Body:

Model (Part 1 and 2):

The model used in specific aim 2 will be identical to specific aim 1. Prior to the performance of the laparotomy baseline cortisol levels, an electrolyte panel, ionized calcium, comblete blood count, prothrombin time, partial thromboplastin time, lactate level and an arterial blood gas will be drawn.

Animals will be randomized. These will include 4 different resuscitation regimens: 1) baseline pre-injury blood pressure, 2) 10 mmHg below the rebleeding blood pressure, 3) 30 mmHg below the rebleeding blood pressure and 4) no resuscitation and 4 different resuscitation fluids: 1) lactated Ringer's, 2) 5% HTS (500 cc followed by LR), 3) hextend and 4)PolyHeme. Each fluid will be studied at baseline, 10 mmHg below the rebleeding point and 30 mmHg below the rebleeding point and there will be a control group that is not resuscitated.

A standardized Grade V liver laceration will be made using the liver clamp. The animals will be allowed to bleed freely for 1 hour. Following this time period, blood will be evacuated from the abdomen and measured. Resuscitation will then be initiated as dictated by randomization and the abdomen will be closed. The investigators will be blinded to the resuscitation fluid and no effort beyond the use of blankets will be made to maintain normothermia. Resuscitation endpoints will be maintained for 24 hours or until death.

Laboratory studies will be obtained hourly for the 1^{st} 4 hours and every 2 hours thereafter. For those animals that expire prior to the 24 hour period, lab studies will be drawn just before death.

Following completion of the study, the animals will be euthanized and intra-abdominal blood loss will be measured. A necropsy will be performed and the liver injury will be graded using the AAST liver injury grading system to insure the injuries are similar between groups. Lung, kidney and liver tissue will obtained for myeloperoxidase and hematoxylin and eosin staining to assess for neutrophil recruitment and tissue injury secondary to shock and resuscitation. Liver tissue will be obtained from non-injured areas. Lung, kidney and liver tissue will also be processed for RNA and protein extraction. Interleukin-6, granulocyte colony stimulating factor and tumor necrosis factor-alpha mRNA will be measured by quantitative reverse transcriptase polymerase chain reaction. Nuclear factor –κB and signal transducer and activator of transcription-3 activity will be measured by electron mobility shift assay. Interleukin-6, granulocyte colony stimulating factor and tumor necrosis factor are important mediators of the systemic inflammatory response following shock and resuscitation. These mediators are present early after resuscitation and they have been implicated in the development of ARDS and MOF. Signal transducer and activator of transcrition-3 and nuclear factor – κB are important transcriptional proteins which amplify the systemic inflammatory response and contribute to organ injury.

In order to establish baseline values for the inflammatory mediators, 2 additional groups will be studied. The 1st group will be a control group of 6 animals that undergo immediate euthanization and harvesting of their organs. This will establish baseline cytokine values in the animal population. The 2nd group will be a sham group of 6 animals that undergoes all of the surgical manipulations described as well as 24 hours of anesthetic but no liver injury. This group will elucidate the cytokine response of the model exclusive of the injury.

Primary outcome variables will be blood loss, mortality, time to death, extent of coagulopathy, resuscitation requirements and acidosis. mRNA and protein studies will be utilized to determine the degree of dysfunctional inflammation present as a predictive model for the development of adult respiratory distress syndrome and multiple organ failure.

Uncontrolled hemorrhagic shock model

In Part 1, Thirty-eight Yorkshire crossbred swine with a mean weight of 35 kg underwent a 16-hour pre-operative fast except water ad libitum. Animals were pre-anesthetized with 8 mg/kg Telazol® (Fort Dodge Animal Health, Fort Dodge, IA) by intramuscular injection, intubated with a 6.5 mm to 7.5 mm oral endotracheal tube, and mechanically ventilated. Adequate anesthesia, assessed by monitoring jaw tone, was maintained with isoflurane (Abbott Laboratories, North Chicago, IL) and adjusted by the animal technician as needed. Respiratory rate was adjusted to maintain an end-tidal CO_2 and Pco_2 of 40 ± 4 mmHg, and tidal volume was set at 12 ± 2 cc/kg. After establishing anesthesia and mechanical ventilation, invasive monitoring devices were placed including an esophageal thermometer, left common carotid arterial catheter, and left external jugular venous catheter. Animal temperature was maintained at 38.0 ± 1.5°C using warmed fluids and external warming devices.

Six swine were randomized to a control arm. These swine were anesthetized and sacrificed immediately to obtain tissue for baseline data. An additional six swine were randomized to a sham surgery arm. After establishing anesthesia and placing monitoring devices, these swine underwent laparotomy, suprapubic Foley catheterization, and splenectomy. The spleen was weighed and lactated Ringer's solution (LR), 3 cc/g spleen weight, was infused. The abdomen was closed with towel clamps. Anesthesia was continued for two hours prior to animal sacrifice and tissue harvesting. Data obtained from the sham animals served as a control for model effects including laparotomy, splenectomy, mechanical ventilation and anesthesia.

The remaining thirty animals were randomized to a no fluid arm (NF) or to one of two blinded resuscitation arms (LR, HEX). After establishing adequate anesthesia and placing invasive monitoring devices, these animals also underwent laparotomy, suprapubic Foley catheterization, splenectomy and splenic volume replacement. Following a 15-minute stabilization period, pre-weighed laparotomy sponges were placed into the pelvis and inferior left and right pericolic gutters. Standardized grade V liver injuries were then created using a specially designed clamp. The clamp was closed twice over the central portion of the liver, producing a consistent injury pattern involving a large amount of parenchymal damage as well as laceration of one or more central hepatic veins. Figure 2 is a representative hepatic injury produced with this clamp. This technique resulted in injuries consistent with grade V injuries defined by the American

Association for the Surgery of Trauma Organ Injury Scaling System. This model has been described in several prior studies.

Animals were allowed to hemorrhage for 30 minutes following injury. However, all animals frank hemorrhage stopped spontaneously before the 30-minute period ended. Active hemorrhage was collected with the pre-weighed sponges and by suction, avoiding disturbance of the liver. The abdomen was then sutured closed. Blood loss was determined by reweighing the sponges and suctioned blood and was reported as a mean for each group in ml/kg ± standard deviation.

After the 30-minute hemorrhage period, animals randomized to the two resuscitation arms received either LR or HEX. Both commercially prepared fluids were unmodified. Fluids were infused as needed to achieve and maintain baseline blood pressure for 90 minutes. An infusion rate of 165 cc/min was chosen because this is one half the rate delivered by a Level I infuser and animals weighed approximately one half the weight of a normal adult human. At the end of the 90-minute resuscitation period, animals were sacrificed and tissues harvested.

Animals randomized to the no fluid arm were maintained under anesthetic conditions identical to resuscitated animals. No fluid animals were sacrificed and tissues harvested 120 minutes after injury. The liver was removed and examined to insure comparable injuries between study arms. Samples of lung tissue were harvested through a left lateral thoracotomy for tissue levels of interlukin-6 (IL-6), granulocyte colony stimulating factor (G-CSF), and tumor necrosis factor alpha (TNF-α) messenger ribonucleic acid (mRNA) and for assessment of neutrophils sequestered in alveolar walls. Lung tissues for mRNA analysis were flash frozen with liquid nitrogen and stored at -80° Celsius. Tissues for histologic analysis of neutrophil sequestration were stored in formalin.

Quantitative Reverse Transcription Polymerase Chain Reaction Analysis

Tissue levels of IL-6, G-CSF, and TNF-α mRNA were determined using quantitative reverse transcriptase polymerase chain reaction (Q-RT-PCR). Total RNA was isolated from flash-frozen tissue using a commercially available kit (RNeasy® Mini Kit; Qiagen Inc., Valencia, CA). RNA (5 ng for 18S and 500 ng for the gene of interest) was reverse-transcribed into cDNA with random hexamers using the SuperScript™ III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA) under the following conditions: 10 minutes at 25ºC, 50 minutes at 50ºC, followed by reaction termination at 85ºC for 5 minutes. Remaining RNA was removed with RNase H at 37ºC for 20 minutes. Quantitative PCR was performed utilizing the TaqMan® Universal PCR Master Mix (Applied Biosystems, Foster City, CA). The endogenous control, 18S ribosomal RNA, was amplified using the Assays-on-Demand primer/probe kit (Applied Biosystems, Foster City, CA). Genes of interest were amplified using custom primers and probes. All reactions were performed on the ABI Prism® 7900HT (Applied Biosystems, Foster City, CA) utilizing the following conditions: Stage 1) 2 minutes at 50ºC, Stage 2) 10 minutes at 95ºC, Stage 3) 40 cycles of 15 seconds of melting at 95ºC followed by DNA synthesis for 1 minute at 60ºC. Primers and probes used for gene specific PCR amplification and quantification of swine IL-6, TNF-α, and G-CSF mRNA were derived from published swine sequences. Primers and probes were used at concentrations of 300 nanomoles and 200 nanomoles respectively.

Neutrophil Sequestration Analysis

Lung tissues fixed in formalin, were processed and embedded in paraffin for neutrophil analysis. 5-micron sections were heated in citrate buffer for 30 minutes. The sections were then incubated with rabbit anti-myeloperoxidase antibody (Dako, 1:1600) for 45 minutes, followed by biotinylated anti-rabbit secondary and avidin/biotin/HRP (Vectastain kit; Vector Labs, Burlingame, CA). Staining was visualized by incubating with DAB for 10 minutes followed by hematoxylin counterstaining and cover-slipping. Two separate areas of lung were sampled for each animal. For each slide, five high-power fields were examined by light microscopy for the presence of neutrophils within the alveolar walls.

In part 2, 62 Yorkshire crossbred swine were randomized. Six animals were randomized to a control group and 6 were randomized to a sham group. The remaining 50 animals were randomized to 5 groups to include normal saline (NS), 3% hypertonic saline (3%), 3% hypertonic saline with dextran (3%D), 7.5% hypertonic saline (7.5%) and 7.5% hypertonic saline with dextran. (7.5%D) The methods in Part 2 of the study were identical to part 1 with mild exceptions. Spleen replacement fluid consisted of normal saline instead of LR. Normal saline was used because all fluids utilized in part 2 contained various concentrations of NaCl. Following the 30 minute uncontrolled hemorrhage period animals were resuscitated with a single 250cc bolus of study solution given over 10 minutes. The rate of study solution delivered was determined after using 12 developmental animals. Non-invasive tissue oxygenation monitoring was implemented using near infrared spectroscopy. (Hutchinson Technology) The near infrared spectroscopy patch was placed in the left groin and tissue oxygenation was measured continuously throughout the study. All other methods were identical to part 1.

Results

Table 1 contains the cytokine expression data from part 1. Data were reported as fold increase above baseline and are presented as mean ± SEM. Data collected for control animals were designated baseline and assigned a value of 1. The HEX resuscitated animals had significantly more transcription of IL-6 mRNA than controls, shams and NF animals ($P < 0.01$). The LR resuscitated animals had increased IL-6 mRNA transcription compared to controls, shams and NF animals but levels failed to reach statistical significance ($P = 0.06$). IL-6 mRNA levels were not different between the LR and HEX resuscitated animals ($P = 0.51$).

G-CSF mRNA transcription was significantly elevated in both fluid resuscitation groups compared to controls, shams and the NF group ($P < 0.04$). There was no difference in G-CSF mRNA transcription between the LR and HEX resuscitation groups ($P = 0.14$).

TNF-α mRNA transcription was also significantly elevated in fluid resuscitated animals compared to controls, shams and no fluid animals ($P < 0.04$). Again, there was no difference in TNF-α mRNA levels between the LR and HEX resuscitated animals ($P = 0.31$). TNF-α mRNA transcription was also elevated in the NF group compared to controls and shams ($P < 0.01$).

Sequestered neutrophil data, reported as mean number of neutrophils per high-power field, are also presented in Table 1. Sham animals had significantly more sequestered neutrophils than control animals and all animals receiving the grade V liver injury had significantly more

sequestered neutrophils than shams or controls. There was no difference in the number of sequestered neutrophils found in any of the injured animals.

Table 1.

	Control	Sham	NF	LR	HEX	p-value		
n	6	6	6	10	10	NF *v.* LR	NF *v.* HEX	LR *v.* HEX
PMNs	6.9 +/-1.3	12 +/-2	22 +/-3	21 +/-7	21 +/-6	0.426	0.353	0.972
IL-6	1 +/-1.6	3 +/-3	1.4 +/-1	15 +/-20	21 +/-19	0.057	0.009	0.508
G-CSF	1 +/-1.0	2 +/-1	4 +/-3	127 +/-163	325 +/-366	0.040	0.021	0.143
TNF-α	1 +/-0.5	2 +/-2	10 +/-2	106 +/-127	167 +/-136	0.039	0.005	0.315

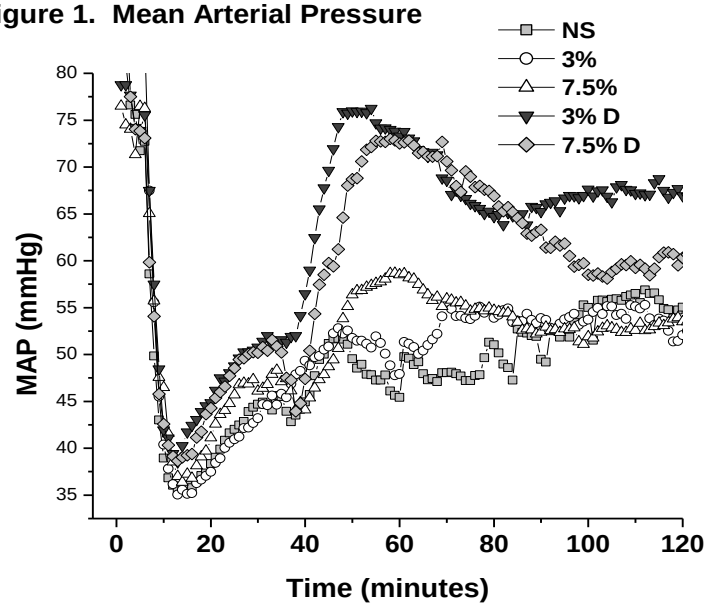
In part 2, Two NS and two 3% animals did not survive to 120 minutes. All animals had similar injuries (2.1 ± 0.9 vessels). Baseline characteristics and end of study data were similar for all groups ($p>0.2$) except for urine output. Despite equal blood loss and resuscitation volumes, animals receiving 7.5% ± D produced 3 to 6 times more urine than animals receiving 3% ± D or NS, *$p<0.03$ (Table 2).

Table 2. Baseline (T0) and end of study (T120) data.

	Wt kg	T0 Temp ºC	MAP T0	1º EBL ml/kg	Nadir MAP	Fluids ml/kg	UOP ml/kg	T120 Temp ºC	2° EBL ml/kg
NS	33±2	37.8±0.6	82±13	23.0±7.0	33±11	7.5±0.5	1.8±2.1	38.0±0.5	1.5±0.7
3%	35±3	37.6±0.5	81±19	23.0±6.6	32±8	7.2±0.8	1.7±1.6	38.0±0.6	1.5±1.1
3%D	34±3	37.9±0.5	81±20	20.8±5.4	34±8	7.3±0.7	2.3±1.1	37.9±0.4	2.0±1.1
7.5%	34±3	37.7±0.5	76±11	21.5±5.4	32±8	7.2±0.8	6.1±4.6*	37.9±0.6	1.6±0.6
7.5%D	34±4	37.7±0.7	79±13	19.3±4.5	35±9	7.6±0.7	6.9±3.6*	37.8±0.5	2.3±0.8

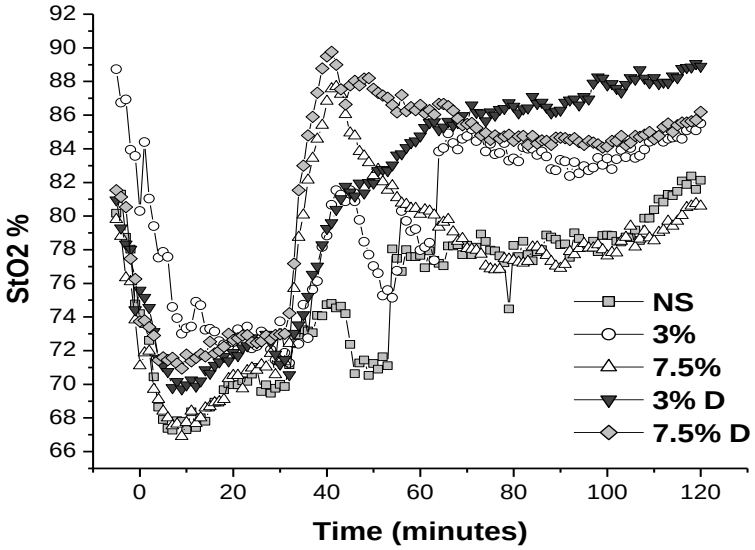
Continuous MAP and StO2 data are presented in Figures 1 and 2. Injuries were created at time zero (T0). All animals experienced a precipitous drop in MAP to similar nadirs followed by a period of auto-resuscitation. The single fluid bolus was administered over 10 minutes beginning 30 minutes after injury. 7.5% saline solutions caused a brief drop in the MAP, more pronounced in the group also receiving dextran. Both HTS solutions containing

Figure 1. Mean Arterial Pressure



dextran produced a significantly greater overall increase in MAP. The 3%D group trended toward a higher MAP at 120 minutes than the 7.5%D group. After injury, a precipitous drop occurred in tissue oxygen saturation, mirroring the drop in MAP. All animals reached similar StO2 nadirs followed by a period of auto-resuscitation. The 4 groups receiving HTS began improving StO2 immediately with fluid administration. 7.5% ± D solutions produced a significantly greater initial increase in StO2. However, this effect began declining within 5 minutes of completing the fluid bolus. The decline was more rapid in the 7.5% group. On the contrary, 3%D continued to improve StO2 over the 90-minute resuscitation period.

Figure 2. Tissue Oxygen Saturation



Baseline aboratory results were similar for all groups. At 120 minutes, platelet count (299±117), PTT (15.5±2.2), PT (14.2±1.3), pH (7.39±0.05), pCO_2 (48±4), pO_2 (481±99), serum lactate (2.4±1.8), and base excess (5.1±3.8) remained similar for all groups. Laboratory data showing significant differences between groups at 120 minutes is listed in Table 3. Groups receiving HTS developed hypernatremia with Na levels peaking 30 minutes after fluid infusion. Serum Na levels remained significantly different at T120. HTS groups also developed significant hyperchloremia. The degree of hypernatremia and hyperchloremia correlated with the infused fluid's NaCl concentration.

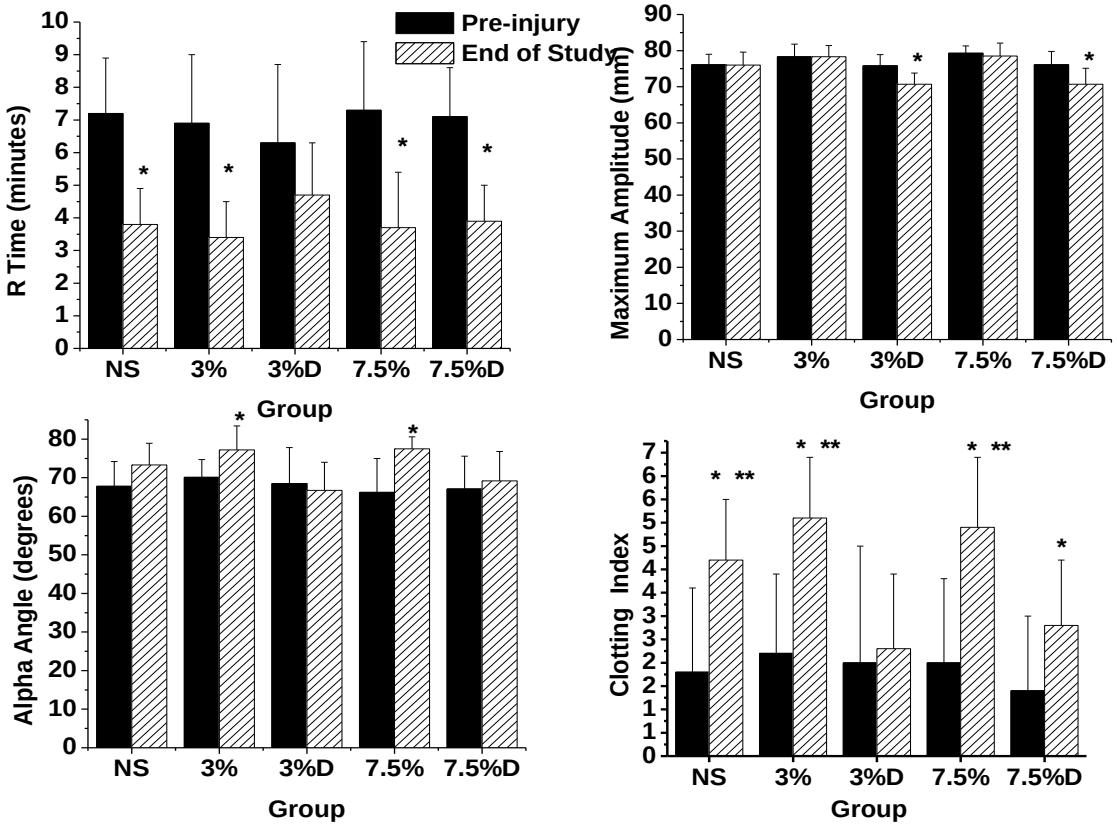
Table 3. End of study (T120) laboratory data.

	Na	Cl	Hct	Fibrinogen	Ur Na
NS	136±1.8	107±3 b,d,e	23.8±3.0 b,d,e	161±47 b	144±3 b,e
3%	139±1 a,b,e	109±4 b,e	22.4±2.7 b,d,e	186±54 b	147±3 b,e
3%D	140±1 a,b,e	111±3 a,b,e	19.6±2.1 a,b,c	150±47	146±3 b,e
7.5%	147±1 a,c,d	118±5 a,c,d	20.7±2.2 a,b	185±51 b	153±5 a,c,d
7.5%D	148±1 a,c,d	120±4 a,c,d	16.7±2.7 a,c,d,e	127±31 c,e	154±3 a,c,d
p<0.05 a *vs.* NS, b *vs.* 7.5%D, c *vs.* 3%, d s*v.* 3%D, e *vs.* 7.5%					

Significant anemia and relative hypofibrinogenemia developed in HTS groups, and was exacerbated by the addition of dextran. HTS groups developed elevated urine Na levels corresponding to serum Na.

TEG data are shown in Figure 3. Reaction (R) time represents the time to onset of clot formation. A significant decrease in R time occurred in all groups except 3%D. The alpha angle represents the rapidity of fibrin buildup and cross-linking. The alpha angle did not increase in animals receiving dextran. Maximum amplitude (MA) is a measurement of clot strength and is affected by platelet number and function as well as by fibrinogen level. The MA decreased significantly in animals receiving dextran. Clotting index (CI) is a calculated measurement of overall coagulation function derived from all measured values. CI increases significantly in all animals except those receiving 3%D

Figure 3. Thrombelastography Data. p<0.05 *v. Pre-injury, **v. 3%D, 7.5%D



As shown in Table 4, resuscitation with 7.5%D results in increased expression of GCSF. This effect is not seen when dextran is not added to 7.5% HTS. Resuscitation with 3%D results in decreased TNF-alpha expression compared to sham animals and equivalent TNF-alpha expression compared to control animals.

Table 4.

Cohort	GCSF	IL-6	TNF-a
Control	1.00 ±2.72 [a]	1.00 ±1.32	1.00 ±0.95
Sham	1.39 ±3.65[a]	2.36 ±4.37	2.19 ±1.70
NS	1.80 ±4.24[b]	12.57 ±16.51	4.42 ±7.46
3S	1.31 ±3.06	6.37 ±9.13	1.61 ±2.74
3D	0.76 ±1.72	8.28 ±11.72	1.01 ±1.42[c]
7.5S	0.66 ±1.50[a]	3.92 ±5.59	0.80 ±1.14
7.5D	2.50 ±5.55	30.17 ±58.10	2.11 ±3.89

[a] $p<0.05$ versus 7.5D, [b] $p<0.05$ versus 7.5S, [c] $p<0.05$ versus sham

Key Research Accomplishments:

1. Aggressive resuscitation following uncontrolled hemorrhagic shock results in dysfunctional inflammation measured in lung parenchyma. The effect is equivalent with lactated Ringer's resuscitation and Hextend resuscitation. This effect is not seen when animals are not resuscitated.
2. Injury and uncontrolled hemorrhagic shock results in increased alveolar neutrophils. This effect is equivalent in animals given no fluid, lactated Ringer's and Hextend.
3. The addition of dextran to hypertonic saline resuscitation solutions results in a more rapid increase in blood pressure following uncontrolled hemorrhagic shock.
4. 7.5% hypertonic saline solutions with and without dextran produce a more rapid elevation in tissue oxygenation than 3% solutions or normal saline.
5. A single bolus of 3% hypertonic saline results in the most persistent elevations of blood pressure and tissue oxygenation over 2 hours compared to other hypertonic saline solutions.
6. Significant hypercoagulability occurred in all animals except for those animals resuscitated with 3% dextran.
7. Resuscitation with 7.5%D results in increased GCSF mRNA expression in the lungs.
8. Following uncontrolled hemorrhagic shock, blood pressure spontaneously rises suggesting that autoresuscitation occurs. This rise in blood pressure correlates with an increase in tissue oxygenation.

Reportable Outcomes

Part 1 of this work was presented at the Northwest Region of the American College of Surgeons Resident's trauma paper competition in December 2004. It was also scheduled for presentation at the Eastern Association for the Surgery of Trauma in January 2005. The abstract was also published in the Journal of Trauma:

Watters JM, Jackson T, Muller PJ, Malinoski D, Todd SR, Schreiber MA. Fluid Resuscitation Increases Inflammatory Response to Traumatic Injury. Journal of Trauma. 2004;57:1378.

The spontaneous increase in blood pressure and tissue oxygenation that occurs after hemorrhagic shock was presented at the 2004 meeting of the Association of Academic Surgeons. The abstract was published in the Journal of Surgical Research:

Differding JA, Watters JM, Muller PJ, Schreiber MA. The Correlation between Mean Arterial Blood Pressure and Tissue Oxygenation after Uncontrolled Hemorrhagic Shock. Journal of Surgical Research. 2004;126:336-337.

The physiologic results of resuscitation with hypertonic saline solutions in Part 2 was the winner of the basic science portion of the Northwest Region of the American College of Surgeons Resident's trauma paper competition in December 2004. This paper was also accepted for presentation at the Western Trauma Association in February of 2005. The abstract was published in the Journal of Trauma as well as the manuscript. The cytokine expression results following resuscitation with hypertonic saline solutions was also presented at the Northwest Region of the American College of Surgeons Resident's trauma paper competition in December 2004. This paper was also accepted for presentation at the Society of University Surgeons meeting in 2005.

Model: Part 3 – Auto-resuscitation in uncontrolled hemorrhagic shock

50 Yorkshire crossbred female swine, weighing a mean of 33 ± 3 kg, underwent a 16-hour pre-operative fast except for water *ad libitum.* All swine were anesthetized with 8mg/kg intramuscular tiletamine hydrochloride/zolazepam hydrochloride (Telazol®, Fort Dodge Animal Health, Fort Dodge, IA), followed by oro-endotracheal intubation, mechanical ventilation, and maintenance of general anesthesia with isoflurane (Abbott Laboratories, North Chicago, IL).

Animals underwent neck cut-down and placement of 16-gauge catheters in both the carotid artery and external jugular vein for invasive blood pressure monitoring, blood sampling, and fluid administration. An InSpectra™ near-infrared, transcutaneous tissue oximeter probe (Hutchinson Technology, Hutchinson, MN) was placed on the inner right hind leg to measure tissue oxygenation (StO_2). Celiotomy was performed, as well as placement of a 16-French Foley suprapubic bladder catheter for monitoring urine output. Splenectomy was performed, and spleen replacement fluid was given using normal saline in a volume three times the mass in grams of the splenectomy specimen, followed by a 15-minute stabilization period. Continuous MAP and StO_2 measurements were recorded starting 5 minutes before the end of the stabilization period. MAP was recorded every 10 seconds using a Digi-Med BPA 400 blood pressure analyzer (Micro-Med, Inc., Louisville, KY), and StO_2 was recorded every 3 seconds using the InSpectra™ monitor.

Using a previously described technique, swine were then given a grade V liver injury with a specially designed clamp, which provided a standardized injury of one or more central hepatic veins. All swine were allowed 30 minutes of uncontrolled hemorrhage. EBL, nadir MAP, and nadir StO_2 were recorded. Blood was sampled prior to splenectomy and at the end of the 30-minute uncontrolled hemorrhage period and was analyzed for pH, base deficit, lactate, and hematocrit. Data were examined during two periods: injury-nadir and nadir-end of study.

Results-Part 3

The mean total EBL was 786 ±190 mL. The mean total blood volume was 75±2 mL/kg, and the mean percentage of total blood volume lost was 32 ±8%. Mean continuous values for MAP and StO_2 are presented in Figure 1. The mean MAP and StO_2 values at various time points in the study are shown in Table 1. There was a significant decrease in MAP during the study, with an overall mean nadir of 33 ±8 mmHg ($p<0.001$ compared to mean MAP at injury). This occurred at a mean of 7:45 ±3:18 min after the liver injury. The StO_2 also significantly decreased to a mean nadir of 65 ±9% ($p<0.001$ compared to mean StO_2 at injury), which occurred at a mean of 11:28 ±9:24 min after liver injury. Pearson's correlation comparing changes in MAP and StO_2 during this period achieved statistical significance (Table 2), but the r-value was less than 0.5.

The relationships between total EBL and the changes in MAP and StO_2 were also investigated during the time from injury to nadir. There was a statistically significant inverse correlation between total EBL and nadir MAP ($r=-0.667$, $p<0.001$), as well as between total EBL and nadir StO_2 ($r=-0.419$, $p=0.002$). However, there was no significant correlation between the total EBL and the drop in MAP ($r=-0.19$, $p=0.18$). Also, the correlation between total EBL and the drop in StO_2, while statistically significant, was weak ($r=-0.285$, $p=0.045$).

During the study period from nadir to 30 minutes after injury, all animals showed a significant increase in both MAP and StO_2 without the administration of any intravenous fluid. The MAP increased to a mean of 47 ±11 mmHg ($p<0.001$), and StO_2 increased to a mean of 71 ±11 ($p<0.001$). Again, the mean changes in MAP and StO_2 showed a significant correlation (Table 2), but the r-value was less than 0.5.

Laboratory studies from baseline and 30 minutes after injury were also significantly different (Table 3). There were significant drops in base excess and hematocrit ($p<0.001$). There was also a significant drop in pH and a significant increase in lactate ($p<0.001$); however, neither was clinically significant.

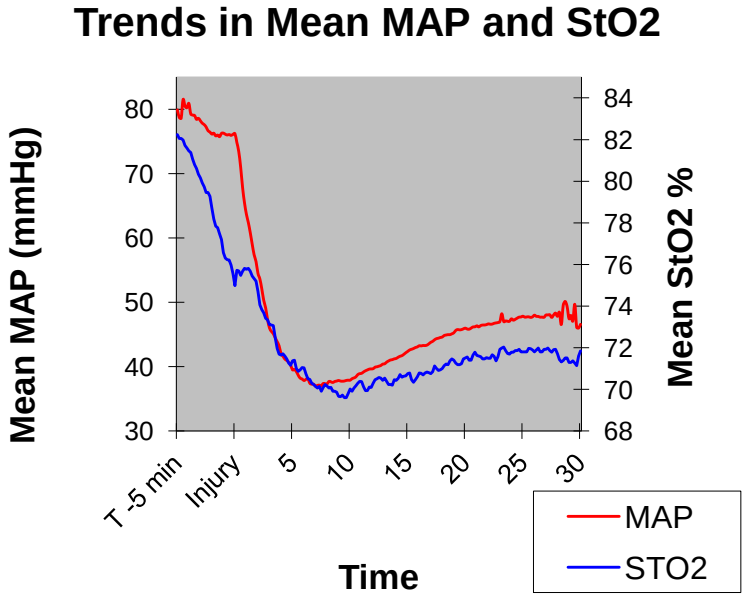


Figure 1. Graph demonstrating changes in mean arterial pressure (MAP) and tissue oxygenation (StO_2) during the study period.

Table 1. Mean values for MAP and StO_2. The nadir values represent the mean of the lowest MAP or StO_2 for each animal during the 30 minute study period.

Study Time Point	Mean MAP (mmHg, ±SD)	Mean StO_2 (%,±SD)
Injury	76 ±17	75 ±10
Nadir	33 ±8[a]	65 ±9[a]
30 minutes	47 ±11[b]	71 ±11[b]

[a] p<0.001 compared to value at injury, [b] p<0.001 compared to value at nadir

Table 2. Mean changes in MAP and StO_2 from liver injury to nadir MAP or StO_2, and from nadir to 30 minutes after injury.

Time Interval	Mean ΔMAP (mmHg ±SD)	p	Mean ΔStO_2 (%±SD)	p	$\Delta MAP/StO_2$ Correlation (r)	p
Injury-nadir	-43 ±15	<0.001	-11 ±8	<0.001	0.42	0.002
Nadir-end study	+14 ±8	<0.001	+6 ±4	<0.001	0.35	0.012

Table 3. Laboratory data, comparing values pre-injury with those obtained at 30 minutes after injury.

Laboratory Parameter	Mean value, baseline (±SD)	Mean value, 30 min. post-injury (±SD)	p
pH	7.46 ±0.07	7.42 ±0.05	<0.001
Base excess (mEq/L)	8.2 ±3.6	4.6 ±3.4	<0.001
Lactate (mmol/L)	1.56 ±0.65	2.61 ±1.41	<0.001
Hematocrit (%)	26.1 ±2.9	22.3 ±2.6	<0.001

Model: Part 4 – Comparison of resuscitation with LR vs. NS

This study was designed to compare hemodynamic and coagulation differences in animals resuscitated with LR and NS. The described liver injury model was utilized. Following 30

minutes of uncontrolled hemorrhagic shock, 20 animals were randomized to receive resuscitation with LR or NS at 165 cc/min to achieve and maintain the baseline blood pressure for 90 minutes. In addition to heart rate, blood pressure and StO2, cardiac output and systemic vascular resistance were measured. Serial coagulation parameters measured included prothrombin time, partial thromboplastin, fibrinogen levels and TEGs (Haemoscope Corp, Skokie Illinois).

Results: Part 4

Ten animals were randomized to each group. One animal in the NS group died just prior to completion of the 2 hour study period. All other animals survived. Table 4 shows the mean initial weight, blood pressure, temperature, vessels injured, blood loss and fluid replacement compared between groups. Despite the fact that the number of vessels injured and initial blood loss were similar between groups, the NS group had greater blood loss following resuscitation and required more than twice the volume of resuscitation fluid to achieve and maintain the baseline blood pressure during the 90 minute resuscitation study period.

		Mean ± Std. Error	Statistical Significance
Survived	**NS**	9 ± .1	0.343
	LR	10 ± .0	
Weight (kg)	**NS**	33.6 ± 1.0	0.165
	LR	35.6 ± .9	
Starting Temp (C°)	**NS**	37.3 ± .6	0.356
	LR	37.9 ± .2	
Baseline MAP	**NS**	70.4±2.7	0.66
	LR	68.6±2.99	
Veins injured	**NS**	1.8±.25	0.382
	LR	1.5±.22	
Spleen replacement fluid (cc)	**NS**	627.0 ± 52.2	0.811
	LR	612 ± 33.2	
EBL after injury per kg	**NS**	22.8 ± 1.9	0.102
	LR	18.5 ± 1.7	
EBL after resuscitation per kg	**NS**	11.6 ± 1.8	0.014 *
	LR	5.2 ± 1.2	
Total EBL per kg	**NS**	34.3 ± 2.9	0.009 *
	LR	23.7 ± 2.1	
Fluids per kg	**NS**	330.8 ± 38.1	0.001 *
	LR	148.4 ± 20.2	

Table 4. Comparison between NS and LR groups of physiologic parameters. (*$p < .05$)

Figure 2 compares MAP between groups during the course of the study. Although, there is a trend toward a lower blood pressure in the NS group at some time points, these differences do not reach statistical significance. Cardiac output and SVR are shown in Figure 3. As the figure shows, resuscitation with NS results in a significant reduction in SVR and elevation of cardiac output. Resuscitation with NS results in decreased tissue perfusion over the course of the study.

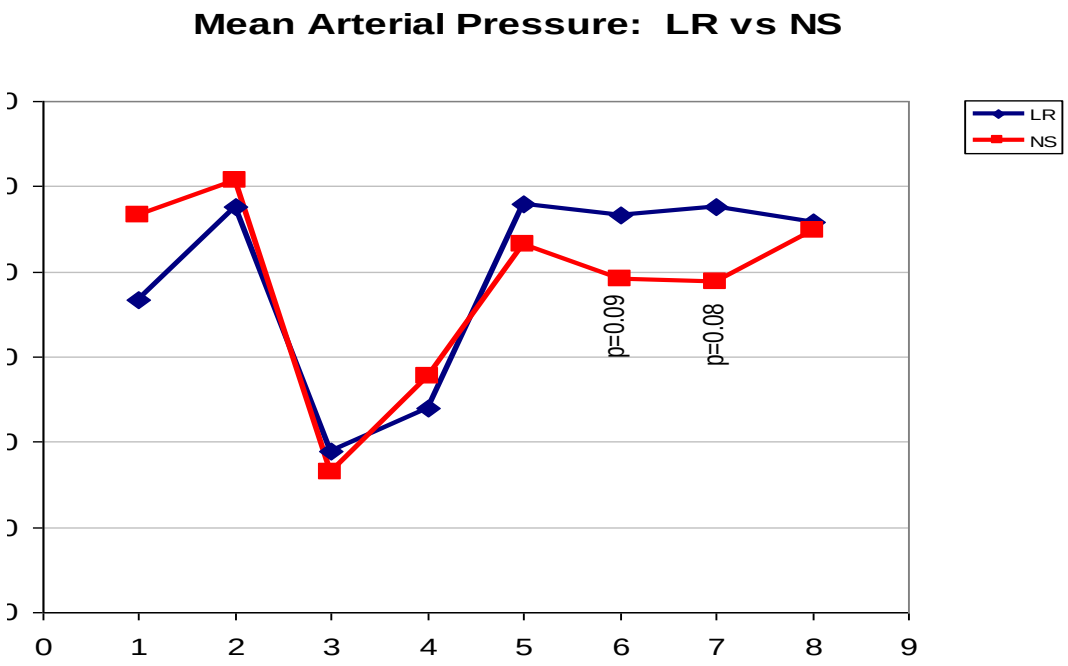


Figure 2. MAP compared between LR and NS groups.

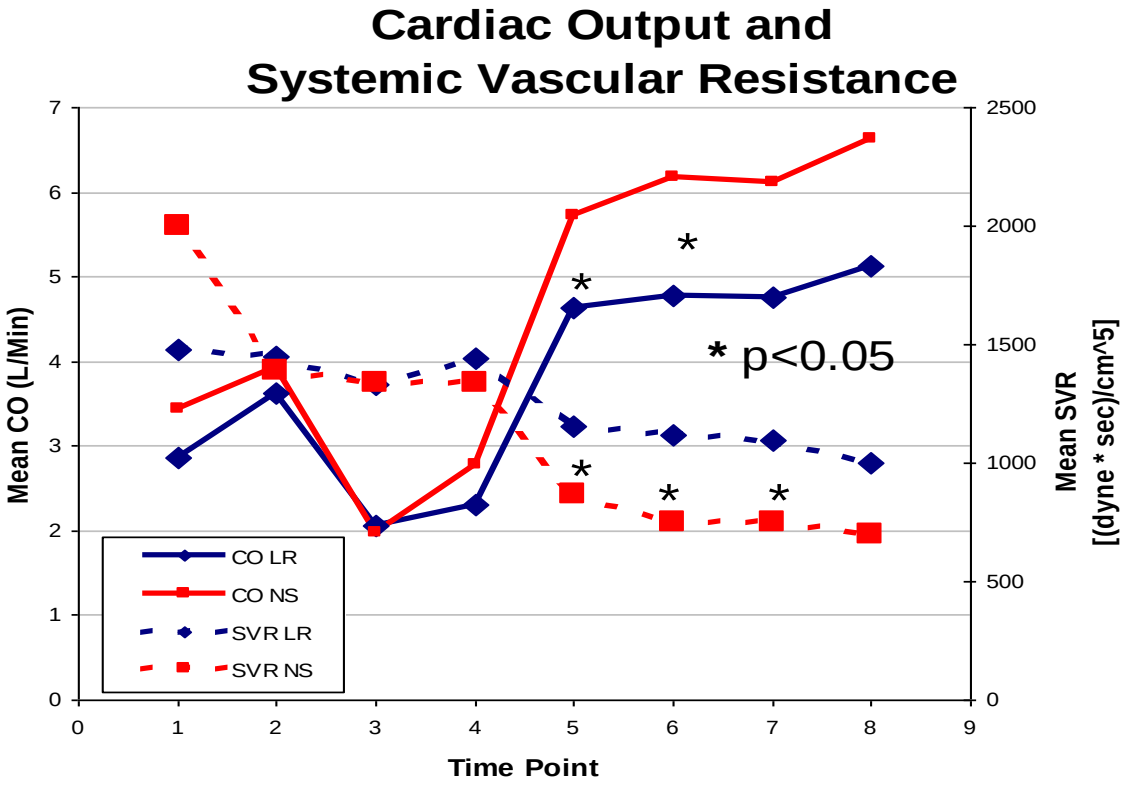


Figure 3. Comparison of CO and SVR between animals resuscitated with LR and NS.

The NS group was significantly more acidotic compared to the LR pigs after resuscitation. (Figure 4) pH was significantly lower in the NS group 30 minutes after injury until the end of study. Interestingly, at this point of the study, the only difference in treatment between the two groups was the equivalent volumes of splenic replacement fluids. The bicarbonate value and base excess were significantly lower 60 minutes after injury and beyond.

Selected laboratory values are displayed in Table 5. The two groups had equivalent hematocrit values at the start of the study. By the end of the study, the NS group had a lower hematocrit. The partial thromboplastin time (PTT) and prothrombin time (PT) were both significantly greater in the NS group compared to the LR group. Fibrinogen was decreased in both groups compared to baseline.

Figures 5-7 show the R value, alpha angle and MA of the two groups. All the parameters showed significant changes during the course of the study. At 60 minutes after injury and beyond, the R value and the alpha angle were significantly different in the LR group as compared to the NS group. At 30 minutes after injury and beyond the MA and CI were significantly higher in the LR group. By the end of the study all of the values in the groups were significantly different from baseline with the exception of the alpha angle in the NS group. These results indicate relative hypercoagulability in both groups but significantly more so in the LR group.

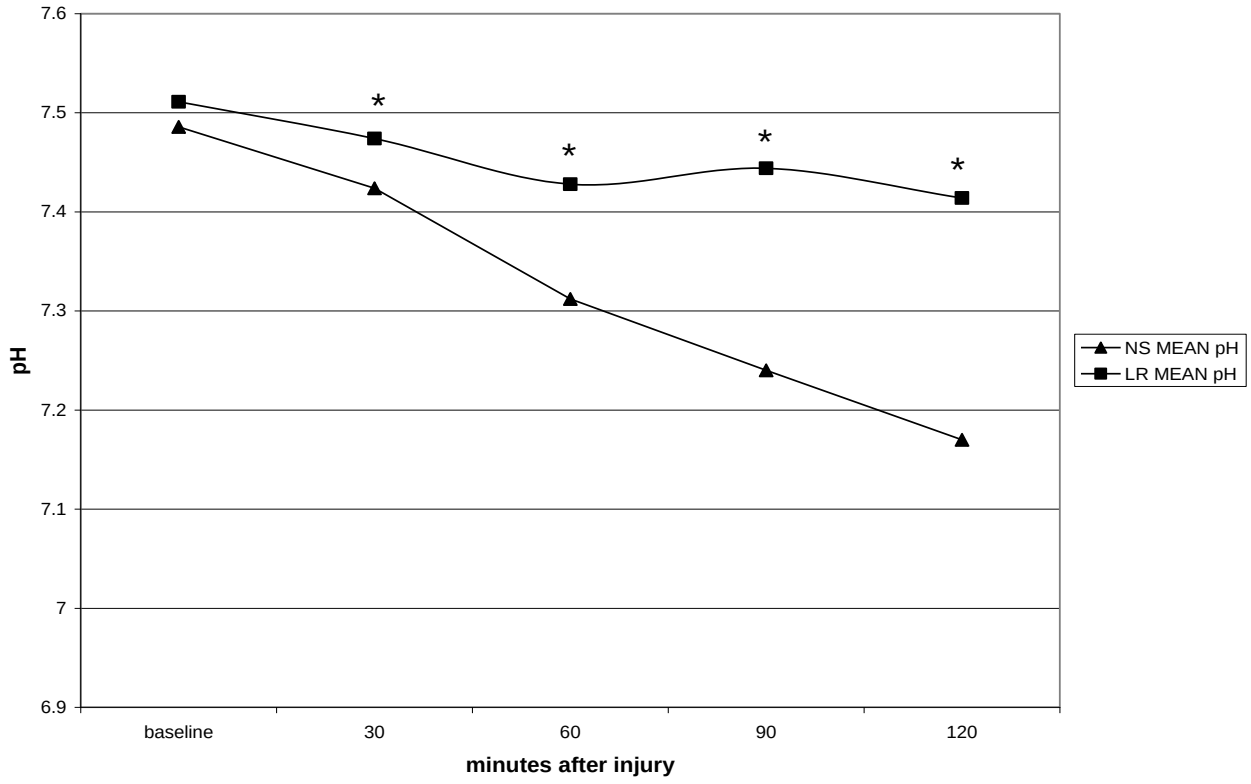


Figure 4. pH values at discrete time intervals after injury in NS and LR groups. * indicates a significant difference ($p < .05$) between groups at that time interval.

		Mean ± Std. Error	Statistical Significance
Baseline Hct	**NS**	26 ± 0.8	0.870
	LR	26.2 ± 0.9	
HCT 120 min post injury	**NS**	12.7 ± 1.1	0.028 *
	LR	16.6 ± 1.2	
Baseline PTT	**NS**	24.2 ± 1.0	0.314
	LR	22.9 ± 0.7	
PTT 120 min post injury	**NS**	25.2 ± 1.1	0.004 *
	LR	21.4 ± 0.5	
Baseline PT	**NS**	13.3 ± 0.2	0.893
	LR	13.2 ± 0.1	
PT 120 min post injury	**NS**	19.0 ± 1.5	0.037 *
	LR	15.5 ± 0.6	
Baseline Fibrinogen	**NS**	149.8 ± 12.2	0.838
	LR	146.1 ± 12.8	
Fibrinogen 120 min post injury	**NS**	68.2 ± 8.2	0.219
	LR	80.5 ± 5.5	

Table 5. Comparison between NS and LR groups of hematologic laboratory parameters drawn at discrete time points. (* signifies statistical significance with $p < .05$)

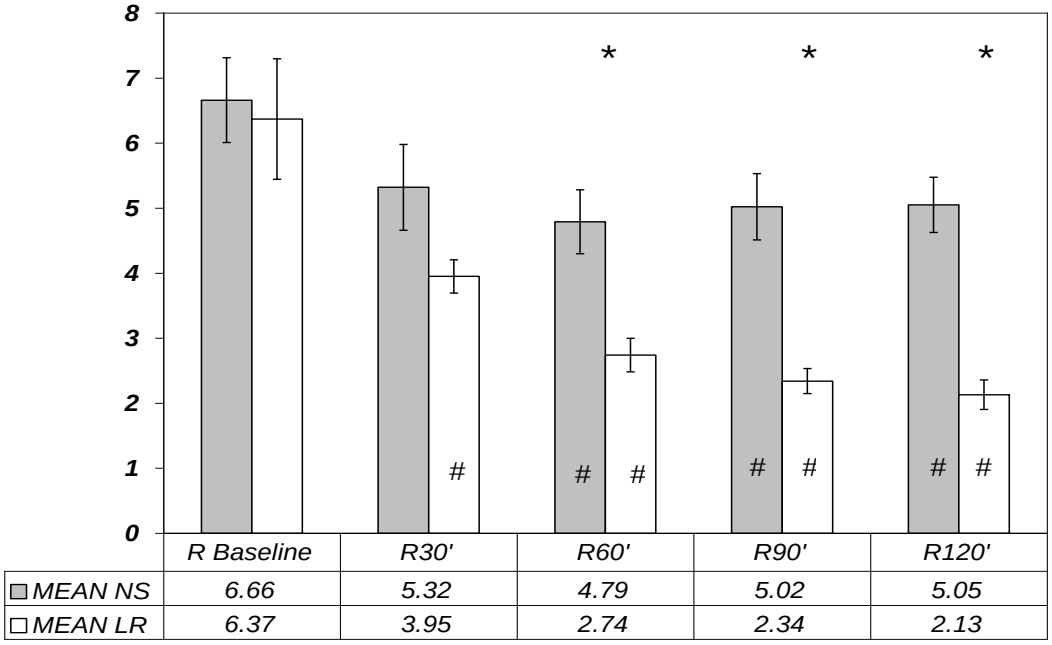
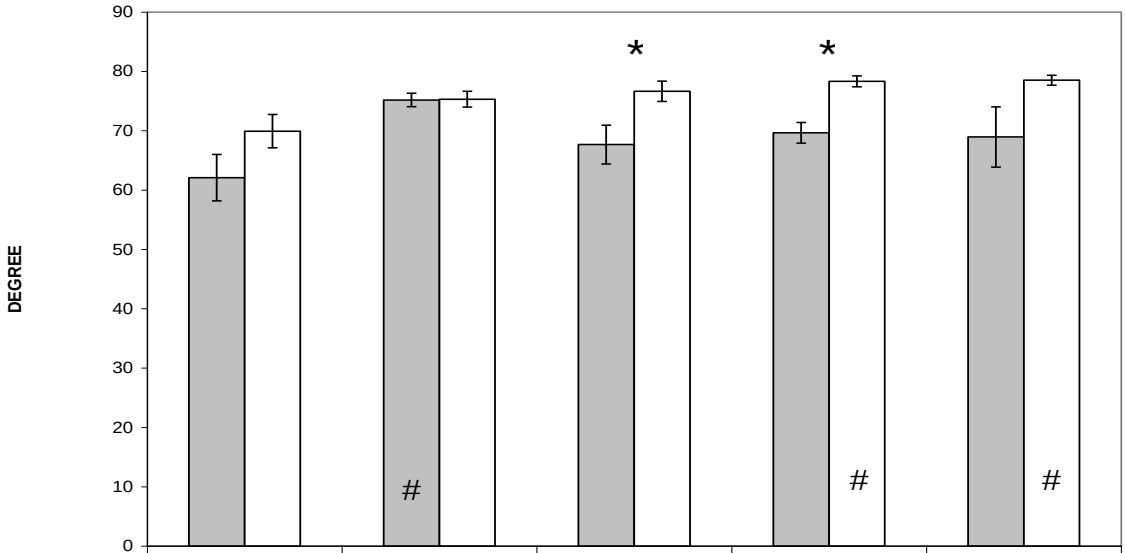
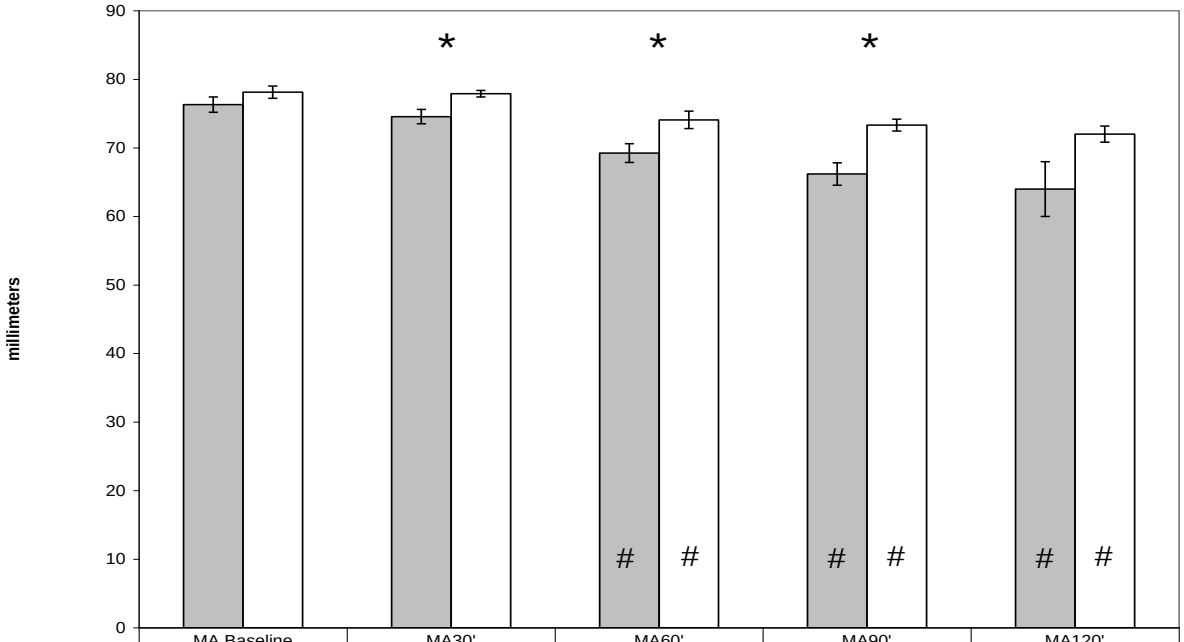


Figure 5. TEG R values at discrete time intervals after injury in NS and LR groups. * indicates a significant difference ($p < .05$) between groups at that time interval. # indicates a significant difference from the baseline value. ($p<.05$)



	ANGLE Baseline	ANGLE30'	ANGLE60'	ANGLE90'	ANGLE120'
MEAN NS	62.1	75.19	67.67	69.65	68.95
MEAN LR	69.93	75.32	76.64	78.34	78.52

Figure 6. TEG Alpha Angle values at discrete time intervals after injury in NS and LR groups. * indicates a significant difference ($p < .05$) between groups at that time interval. # indicates a significant difference from the baseline value. ($p<.05$)



	MA Baseline	MA30'	MA60'	MA90'	MA120'
MEAN NS	76.32	74.56	69.23	66.18	63.99
MEAN LR	78.12	77.90	74.08	73.31	72.01

Figure 7. TEG MA values at discrete time intervals after injury in NS and LR groups. * indicates a significant difference ($p < .05$) between groups at that time interval. # indicates a significant difference from the baseline value. ($p<.05$)

Key Research Accomplishments

1. Following uncontrolled hemorrhagic shock, MAP spontaneously increases in the absence of resuscitation.This increase in MAP is associated with increases in tissue oxygenation.
2. Change in MAP correlates with change in StO2 in uncontrolled hemorrhagic shock.
3. The addition of dextran to 7.5% hypertonic saline results in increased dysfunctional inflammation.
4. In a Grade V liver injury model of uncontrolled hemorrhagic shock, twice the volume of NS is required to resuscitate to the same blood pressure end point as LR.
5. Resuscitation of uncontrolled hemorrhagic shock with NS results in increased cardiac output and decreased systemic vascular resistance compared to LR.
6. Resuscitation of uncontrolled hemorrhagic shock with NS results in less hypercoagulability than resuscitation with LR.

Reportable Outcomes

Part 3 of this work was presented at the 2004 meeting of the American Association of Surgery. The abstract was published in the Journal of Surgical Research.

Part 4 of this work was presented at the 2005 meeting of the Oregon Chapter of the American College of Surgeons and at the 2005 meeting of the Portland Surgical Society. This work was also accepted for presentation at the 2006 meeting of the Eastern Association for the Surgery of Trauma. The abstract was published in the Journal of Trauma as well as the manuscript.

Part 5 – Hemodynamic Effects of lactated Ringer's and normal saline.

This was a randomized controlled trial using twenty female Yorkshire crossbred pigs. The pigs underwent a 16-hour pre-operative fast except for water ad libitum and were pre-anesthetized with an intramuscular injection of 8 mg/kg Telazol® (Fort Dodge Animal Health, Fort Dodge, Iowa). They then underwent oral tracheal intubation with a 7.0 mm or 7.5 mm endotracheal tube and were placed on mechanical ventilation. Respiratory rate was adjusted to keep pCO2 values between 40-50 torr. Anesthesia was maintained using 2% isoflurane in 100% oxygen. An esophageal thermometer was inserted.

Animal temperature was controlled utilizing external warming devices. Once the swine were anesthetized, left cervical cut downs were performed and a central venous polyethylene catheter was inserted into the external jugular vein. The venous line was used for administration of the resuscitation fluids. Femoral artery cut down was performed to place a 4-F aortic catheter with an integrated thermistor tip (Pulsion Medical Systems, Munich, Germany) for continuous blood pressure monitoring and blood sampling. Mean arterial pressure (MAP) and heart rate (HR) were continuously recorded using PiCCO-Technology that was connected to the PiCCO *plus* monitor (Pulsion Medical System, Munich, Germany).

The PiCCO technology system allows hemodynamic monitoring through two different techniques, either intermittently by transpulmonary thermodilution or continuously by pulse contour wave analysis. It is a validated, less invasive alternative to the Swan-Ganz catheter for

the measurement of cardiac output (CO). For transpulmonary thermodilution, a bolus (15 ml per bolus) of cooled (0-6°C) crystalloid fluid was injected through a venous catheter and the thermistor tipped arterial catheter placed through the femoral artery would measure the subsequent temperature changes. These measurements were done manually and randomly throughout the respiratory cycle to obtain CO and SV measurements and calculated systemic vascular resistance values. Thermodilution is also used for calibration of the pulse contour method for continuous measurements of stroke volume (SV) and CO. Using this technology, several other parameters can be measured to include extravascular lung water and pulse pressure variation. Increases in extravascular lung water have been correlated with the development of ARDS.

The animals underwent a midline celiotomy, suprapubic Foley catheter placement, and splenectomy. Splenectomies are performed in swine hemorrhage models because of the spleen's distensibility and the resultant variation in amounts of sequestered blood. The spleen was weighed and, based on randomization, either LR or NS was infused to replace three times the spleen weight. Cystostomy was performed and a foley catheter was placed to measure urine output. The abdomen was then closed with towel clamps.

Following a 15-minute stabilization period, the abdomen was opened and residual peritoneal fluid was removed. Pre-weighed laparotomy pads were placed in both paracolic gutters and the pelvis to facilitate blood collection. A standardized Grade V liver injury (injury to a central hepatic vein) was created with a specially designed clamp. The clamp was positioned in the middle of the liver, placing the right hepatic vein, the left hepatic vein, and the portal vein at risk for injury. This technique resulted in injuries consistent with grade V injuries defined by the American Association for the Surgery of Trauma Organ Injury Scaling System. This model has been described in several prior studies. The time of injury was considered the start time of the two-hour study period. Following 30 minutes of uncontrolled hemorrhage, the initial blood loss, measured by wall suction and the pre-weighed laparotomy pads, was determined. The abdomen was then closed.

We blindly randomized (using a random numbers table) the swine to receive either NS or LR resuscitation at 165 ml/min. This rate is approximately one half the rate delivered by the Level 1 rapid infuser® as the animals were approximately one half the weight of an average human. Resuscitation fluid was administered to achieve and maintain the baseline MAP for 90 minutes post-injury.

Upon completion of the 2-hour study period, the abdomen was reopened and the secondary blood loss was determined by adding the volume of intra-abdominal blood to the weight of the intra-abdominal blood clots. Following the completion of the study the animals were sacrificed by exsanguination. To ensure comparable injuries between the study groups, we removed the liver and identified the number of hepatic vessels injured.

Results - Part 5

One animal in the NS died prior to completion of the study. Table 1 shows mean weight, baseline MAP, vessels injured, spleen replacement fluid, blood loss, urine output and resuscitation volume between the 2 groups.

Table 1. Comparison of LR and NS groups.

Parameter	Study Fluid	Mean ± Std. Error	Statistical Significance
Survived	NS	9	0.343
	LR	10	
Weight (kg)	NS	33.6 ± 1.0	0.165
	LR	35.6 ± 0.9	
Baseline MAP	NS	70.4±2.7	0.66
	LR	68.6±3	
Veins injured	NS	1.8±.25	0.382
	LR	1.5±.22	
Spleen replacement fluid (ml)	NS	627 ± 52	0.811
	LR	612 ± 33	
EBL after injury (ml)	NS	763 ± 65	0.182
	LR	649 ± 50	
Resuscitation volume received (ml)	NS	10901 ± 1208	0.001 *
	LR	5175 ± 622	
Urine output (ml)	NS	1459 ± 280	0.021 *
	LR	652 ± 124	

Injuries and blood loss were similar between groups. Animals receiving NS had twice the fluid requirement and significantly increased urine output. Despite infusion at 165ml/min, we were unable to maintain the pre-injury MAP in some of the NS pigs toward the end of the resuscitation period. Table 2 shows the pH and lactate levels at baseline and 30 minute time intervals until the end of study. Despite similar pH levels at baseline, the NS animals were found to be more acidotic from 30 minutes following injury to the end of the study compared to the LR animals ($p<0.05$).

Table 2. pH values compared throughout the study.

	Study Fluid	Baseline	T30	T60	T90	T120
pH	NS	7.49±0.03	7.42±0.01*	7.31±0.02*	7.24±0.02*	7.17±0.03*
	LR	7.51±0.01	7.47±0.01	7.43±0.01	7.44±0.01	7.41±0.01
Lactate (mmol/L)	NS	1.73±0.14	2.36±0.30	1.71±0.25*	1.34±0.29*	1.30±0.28*
	LR	2.43±0.30	3.25±0.41	5.47±0.60	4.82±0.37	5.99±0.70

Figure 1 shows the average MAP of the groups. MAP in the NS group was lower during the resuscitation phase despite receiving twice the infused volume which was significant from 66-97 min following injury. The mean values for the two groups again become more similar at the final time point. This is in large part due to the death of one pig in the NS group after time point 6. The pig that died prior to the end of the experiment was included in the analysis up until the point at which it died.

Figure 1. Comparison of MAP between LR and NS groups throughout the study.

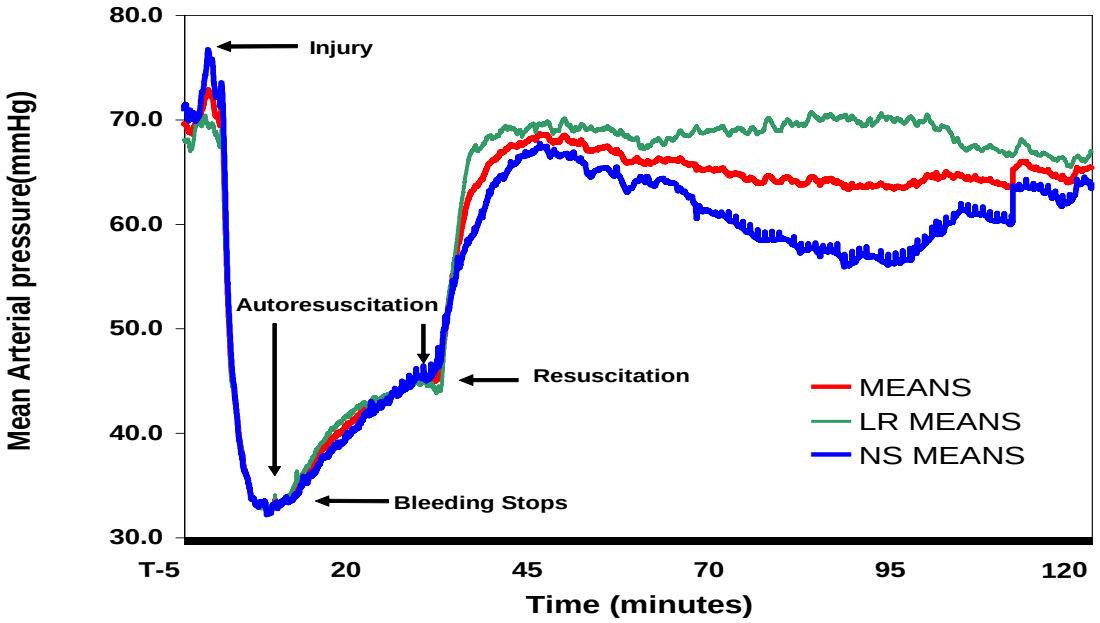


Figure 2 shows etravascular lung water compared between groups. It is notable for the fact that EVLW is immediately increased in the NS group even when resuscitation volumes are similar. This controls for the fact that NS animals received a significantly greater fluid resuscitation compared to LR pigs. This suggests that NS is more likely to predispose patients to the development of ARDS.

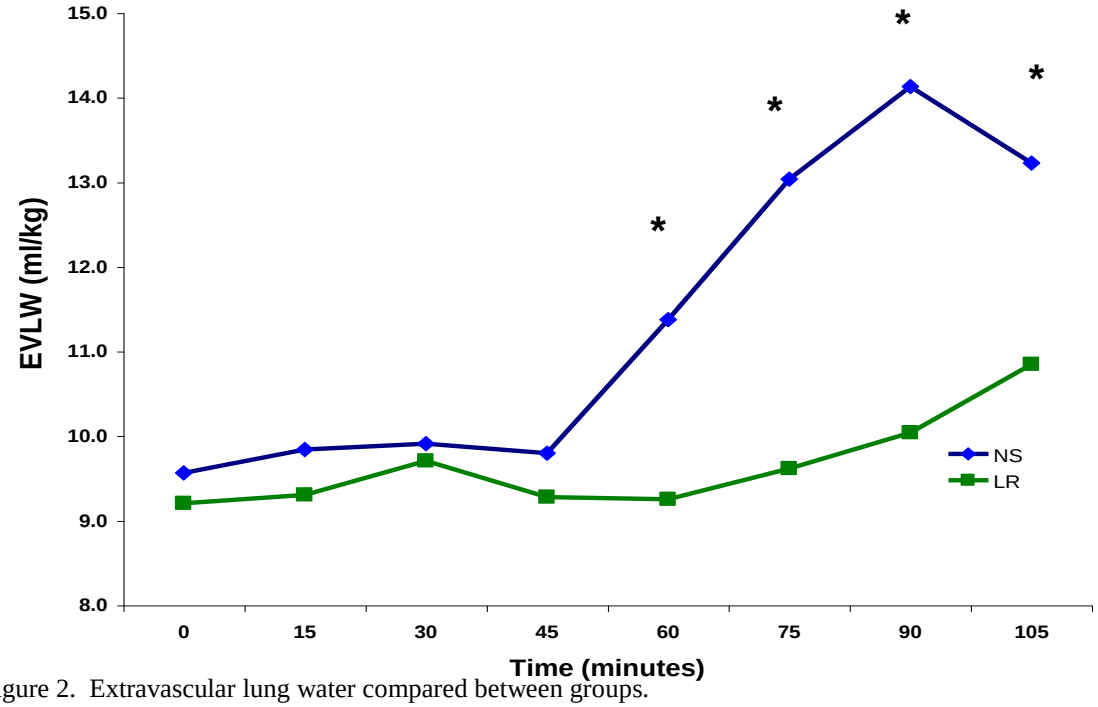
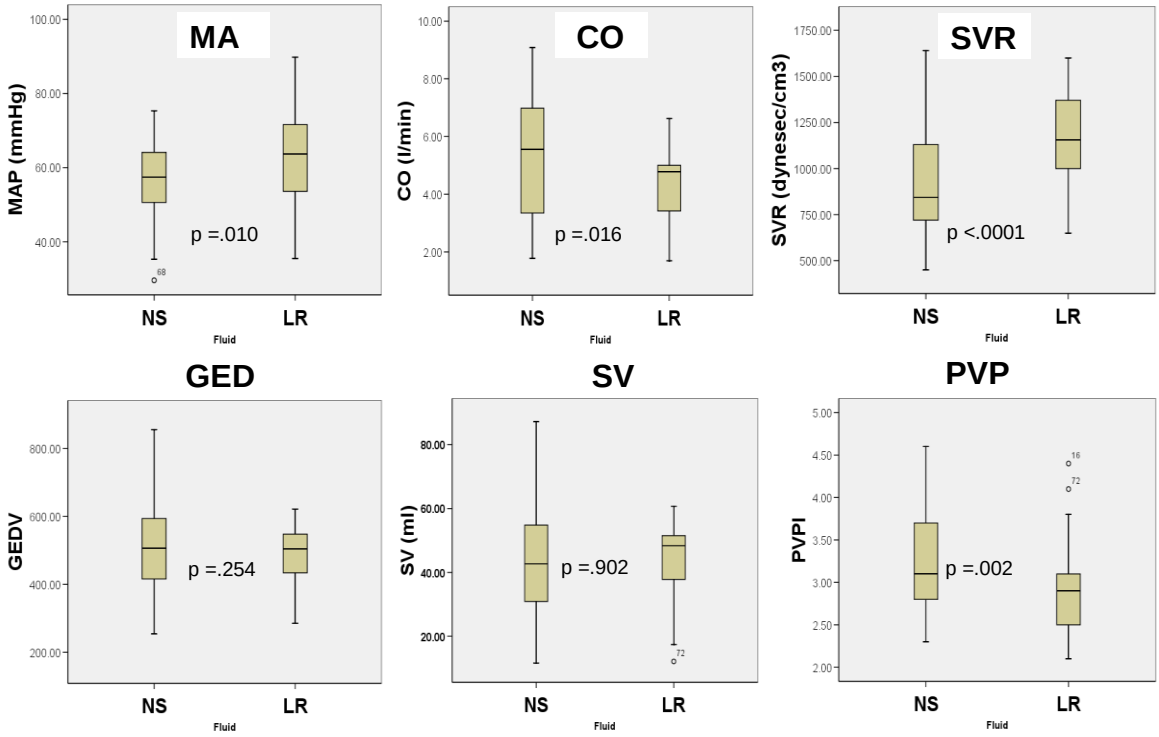


Figure 2. Extravascular lung water compared between groups.

The effect of the fluid resuscitation regimens on hemodynamic parameters is shown is Figure 3. As Figure 3 reveals, NS resuscitation results in marked reduction in SVR and increase in cardiac output. Stroke volume and global end diastolic volume remain similar between the groups. We hypothesize that these hemodynamic changes are a result of acidosis in the NS group.

Figure 3. Comparison of Hemodyamic parameters



Key Research Accomplishments

1. Following uncontrolled hemorrhagic shock resuscitation with lactated Ringer's and normal saline result in differing hemodynamic outcomes.
2. Resuscitation with NS results in decreased SVR and increased CO.
3. Resuscitation with NS also results in increased extravascular lung water independent of the volume of fluid given.

Reportable Outcomes

This work was presented at the 2006 American College of Surgeons Surgical Forum session. The manuscript has been submitted to the Journal of the American College of Surgeons. An additional abstract describing extravascular lung water was been submitted to the European Shock Society in Brussels.

Specific Aim 3 - Development of a severe multi-system trauma model that replicates the lethal triad

Yorkshire swine were anesthetized with isoflurane, intubated and instrumented for monitoring. The anesthesia and line placement has been previously described. A femur fracture and soft tissue injury was then created in the area of the left groin utilizing a captive bolt gun. Following this injury, animals underwent a 60% controlled total blood volume hemorrhage. Mean arterial pressure was monitored continuously throughout this period and if the blood pressure dropped to less than 25 mmHg, hemorrhage was stopped and animal were resuscitated with NS to a blood pressure of 30 mmHg. Animals underwent a celiotomy and placement of a suprapubic catheter. Utilizing cooled fluids their temperature was lowered to 33C. Following controlled hemorrhage there was a 30 minute shock period. The hemorrhage volume was then replaced with normal saline given in a 3:1 ratio. Hemodynamic parameters were measured continuously. Thrombelastography (TEG), PTT, PT and laboratory values were collected at baseline, after the shock period and after NS replacement. Animals then underwent Grade V liver injury as has previously been described. Thirty seconds after injury the livers were packed.

Following creation of this model, at OHSU it was replicated at the Institute of Surgical Research and Harvard. Reproducibility of the model was compared between the 3 centers to determine if a multi-center study could be performed.

Results

Twenty-nine animals were used to complete the initial comparison of the groups. 5 animals (17%) died before completion of the study period. Mean arterial pressure after the shock period was 32±2 mm Hg and was similar between centers ($p=0.4$). Mean pH, base excess, and lactate levels were 7.29±0.02, -8.20±0.65 mmol/L, and 5.29±0.44 mmol/L, respectively, following NS replacement. This was not different between centers ($p>0.05$). PTT, PT, and TEG R' values were different ($p<0.01$). Similar spun hematocrit levels were achieved following controlled hemorrhage ($p=0.15$) and dilution ($p=0.9$).

Key Research Accomplishments

1. A severe multi-system shock model which reliably reproduces the lethal triad can be created in swine with good survivability.
2. This shock model can be reproduced at other centers with comparable results except for coagulation parameters.

Reportable Outcomes

Specific Aim 3 was presented at the 2007 ATACCC meeting and the 2007 Shock Society meeting. This work was also presented the 2007 Region X Residents' Competition of the American College of Surgeons Committee on Trauma. The manuscript was submitted and published in the journal Shock.

Specific Aim 4 – Optimal Hemostatic Resuscitation

The model was developed at the Oregon Health and Science University, Portland, Oregon (center 1), and exported to the United States Army Institute of Surgical Research (USAISR, center 2) and Massachusetts General Hospital/Harvard Medical School (MGH, center 3).

We developed a complex, combat-relevant, multisystem injury model of liver injury, long bone fracture and soft tissue injury, and hemorrhagic shock with hypothermia and acidosis. We then simulated an injury phase, a preoperative phase (including prehospital care, transport and emergency department), and an operative phase of resuscitation. (Figure 1)

Study protocol
Thirty-seven female Yorkshire crossbred swine were utilized. Animals were delivered 7-10 days prior to the experiment in order to minimize the stress of transport and subsequent potential changes in sympathetic output or inflammatory mediators. An overnight fasting period was observed with the exception of water ad libitum. All animals were ordered such that their weight at the time of the experiment was 39.7 ± 1.1 kg (mean ± SEM). No attempt was made to use a single vendor, and each center made their own arrangements for procurement of animals according to their standard sources.

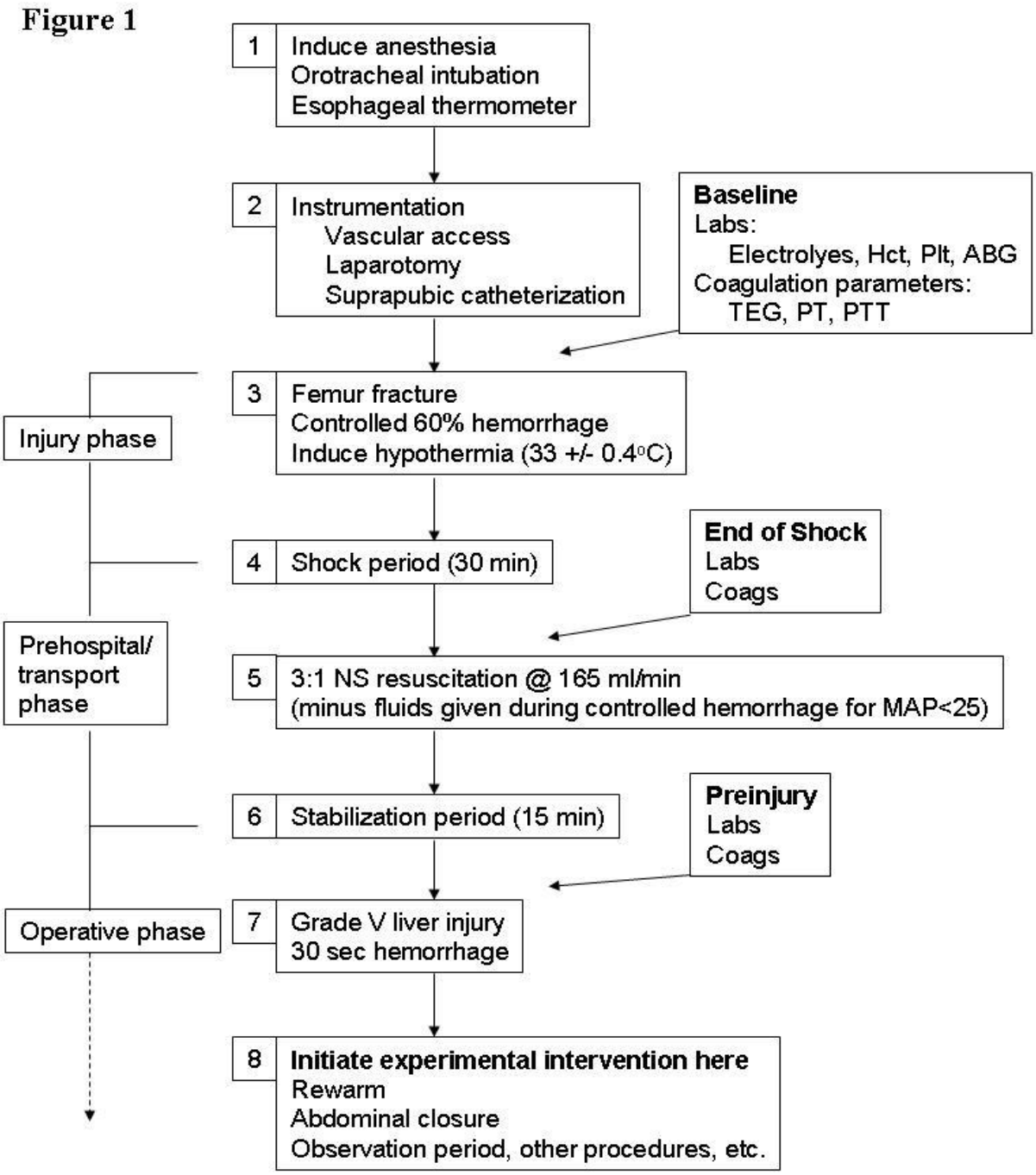
Anesthesia
Anesthesia was induced with 8 mg/kg Telazol® (tiletamine hydrochloride 50 mg/ml, zolazepam hydrochloride 50 mg/ml, Fort Dodge Animal Health, Fort Dodge, Iowa) intramuscularly and isoflurane at 1-3% inhaled. Orotracheal intubation was performed after which an esophageal thermometer was placed. Throughout the study anesthesia was maintained to the clinical endpoints of reflexes and muscle relaxation as is done in humans.

Monitoring, access and pre-experiment procedures
Vascular access was established via neck cutdown and placement of carotid artery and external and internal jugular vein catheters. The femoral artery was cannulated for blood pressure monitoring. Baseline labs were collected and included electrolytes, lactate, spun hematocrit (Hct), activated clotting time (ACT), platelets (Plt), prothrombin time (PT), partial thromboplastin time (PTT), and arterial blood gas (ABG). In addition, a baseline thrombelastogram (TEG, Haemoscope Corporation, Niles, IL) was performed. A celiotomy was then performed, at which time a suprapubic bladder catheter was placed to monitor urine output.

Injury phase
After needle localization, a captive bolt gun was used to fracture the femur and create a soft tissue injury at the midshaft of the left femur. Figure 2 is a 3-D computed tomography (CT) reconstruction of a typical femur fracture created in a study animal by these methods. A controlled hemorrhage was then initiated to remove 60% of the blood volume based on a published, standard equation relating blood volume to body weight for domestic swine. During this period if the mean arterial blood pressure (MAP) fell below 25mm/Hg, normal saline (NS) was infused at a rate of 165 ml/min to keep the MAP>25 mm/Hg. The animal was then cooled to 33 +/-0.4^{o}C using cooled intraperitoneal lavage with crystalloid as needed (most of the animals developed a degree of hypothermia spontaneously due to shock and infusion of IV

fluids). These procedures were followed by a 30-minute shock period, representing time in the field prior to medical intervention.

Figure 1

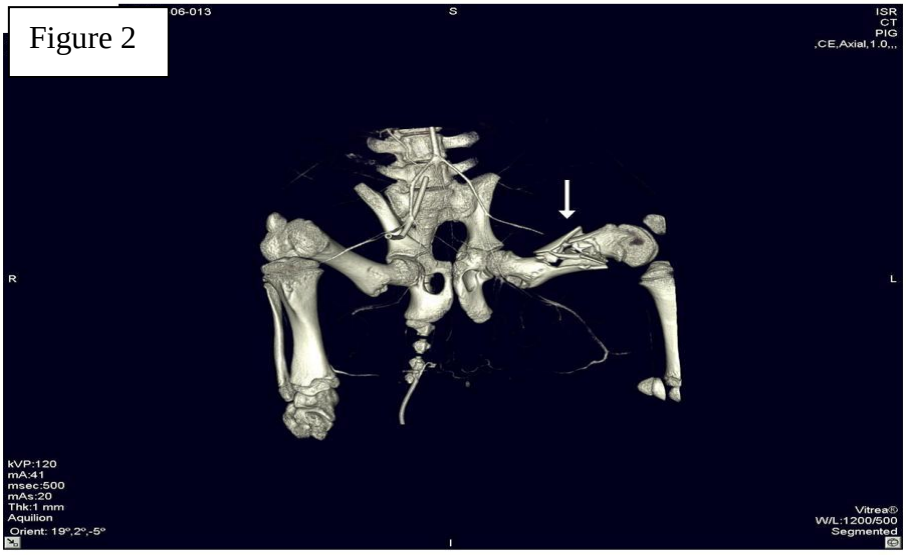


Prehospital care/transport phase
After the 30-minute shock period, electrolytes, spun hematocrit, ACT, PT, PTT, platelets, ABG, and TEG were again recorded. After coagulation studies and lab collection, the hemorrhage volume was replaced with a 3:1 ratio of NS infused at a rate of 165 ml/min, minus any given during the controlled hemorrhage. This reflects current civilian prehospital resuscitative practices.

Operative phase
Following NS resuscitation, a 15-minute stabilization period was observed, during which a baseline MAP was recorded and preweighed laparotomy sponges were placed in both paracolic gutters and in the pelvis for blood collection. Labs and coagulation studies were again collected, and a previously described grade V liver injury was created at the confluence of the right and middle hepatic veins using a specialized clamp. Thirty seconds of hemorrhage were then followed by evacuation of blood from the abdomen and packing of the liver with a fixed number of additional preweighed laparotomy sponges. The liver injury was designed to provide a second stressor after initial injury and also to create a standardized injury that had the potential to rebleed, both of which simulate a laparotomy after trauma in a patient with solid organ injury. Thirty seconds after injury, the liver was packed with laparotomy sponges in a standardized fashion. Randomized treatments were initiated at the same time as packing was initiated. Randomization groups included controls (no resuscitation), whole blood, 1:1 FFP:PRBCs, FFP and Hextend. A sham group underwent all surgical procedures to include femur fracture, exploratory laparotomy and line placement but did not undergo controlled hemorrhage, liver injury or resuscitation. The volume of the treatment resuscitation was equivalent to the blood loss from the controlled hemorrhage.

Study Variables
Physiologic variables included survival, MAP, blood loss from the controlled hemorrhage, and blood loss due to the liver injury. Laboratory values include Hct, lactate, Plt, ABG, and electrolytes. Coagulation parameters include the PT, PTT, ACT, and TEG values.

Figure 2



Results

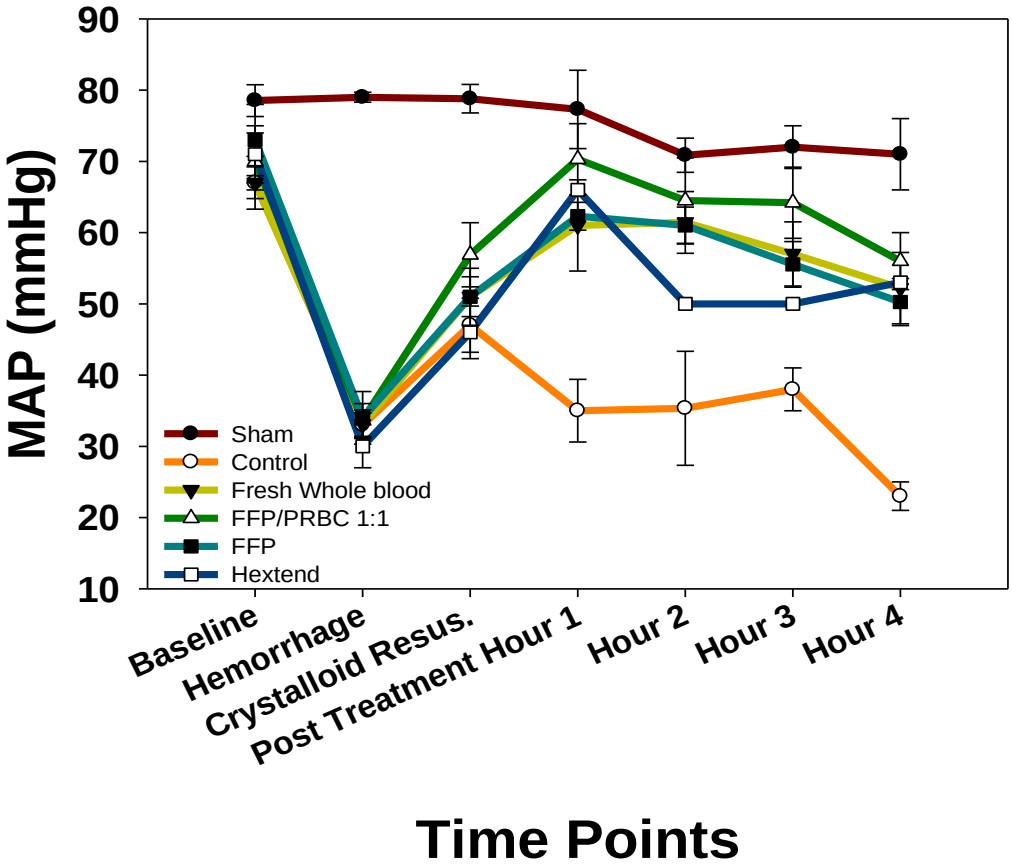
Eight of the animals died during the model period, for a mortality rate of 21.6%. Animals that died prior to the completion of the model period (liver injury and 30-second blood loss) were excluded from the analysis in that all data points could not be collected. Mortality was 85% in the control group, 80% in the Hextend group and 0% in each of the blood component groups ($p < 0.05$).

Physiologic variables

Hypothermia was achieved during the shock period, with a pre-liver injury temperature of 33.1 ±0.07°C. Blood loss from the controlled hemorrhage, a function of the calculated blood volume, was 1708 ±35.6 ml or 43.2 ± 0.3 ml/kg body weight.

Mean arterial pressures across groups are shown in Figure 3. Prior to randomization of care animals in all injury groups had a similar physiologic profile with an acute drop in blood pressure, followed by autoresuscitation. MAPs did not differ significantly between surviving animals in the injury groups at the end of the study. Standard error bars reveal minimal variation between centers.

Figure 3. Mean arterial pressures across groups



Laboratory variables

Baseline, end of shock, and pre-injury hemoglobin values were similar between centers ($p>0.10$). A significant decrease in Hgb was seen over the three study phases. The change in Hgb between baseline and end of shock was significant ($p<0.0001$), as was the change in Hgb from the end of shock to pre-injury ($p<0.0001$). Animals in the Hextend and FFP groups had significantly lower Hgb levels at the end of the study compared to animals in the 1:1 group and the whole blood gropup. Figure 4 illustrates these results.

Lactate and base deficit (BD) were different among the three centers at the baseline and end of shock periods. By pre-liver injury, after resuscitation with NS, all BD and lactate values were similar among the three centers. At pre-injury, mean overall BD was 8.2 ±0.65 mEq/L and the mean overall lactate was 5.3 ±0.44 mmol/L. The BD increased at each specific center between baseline and pre-injury ($p<0.0001$). Lactate values increased at each center from baseline to pre-injury ($p<0.0001$). At the end of the study, lactate levels were significantly higher in animals receiving FFP and Hextend. Figure 5 illustrates these results.

Figure 4. Hemoglobin levels throughout the study

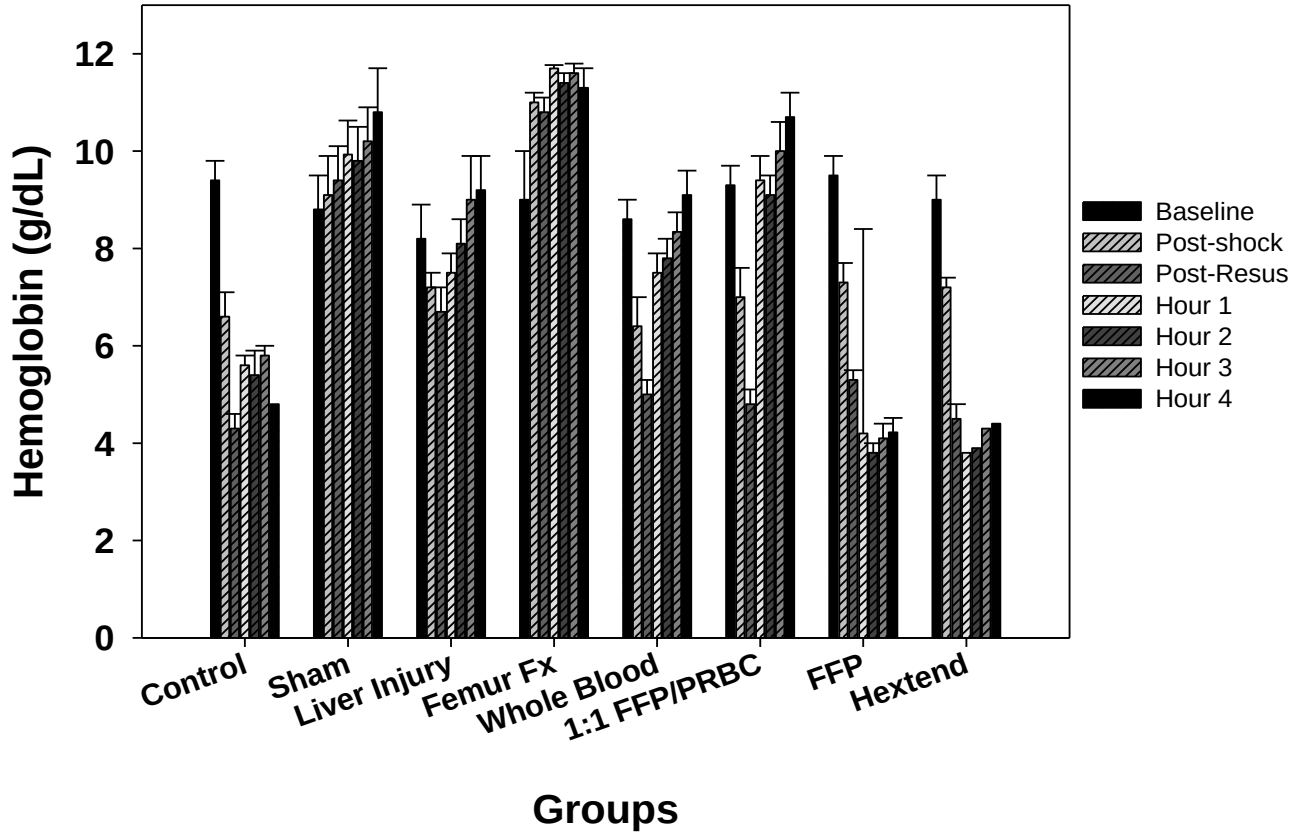
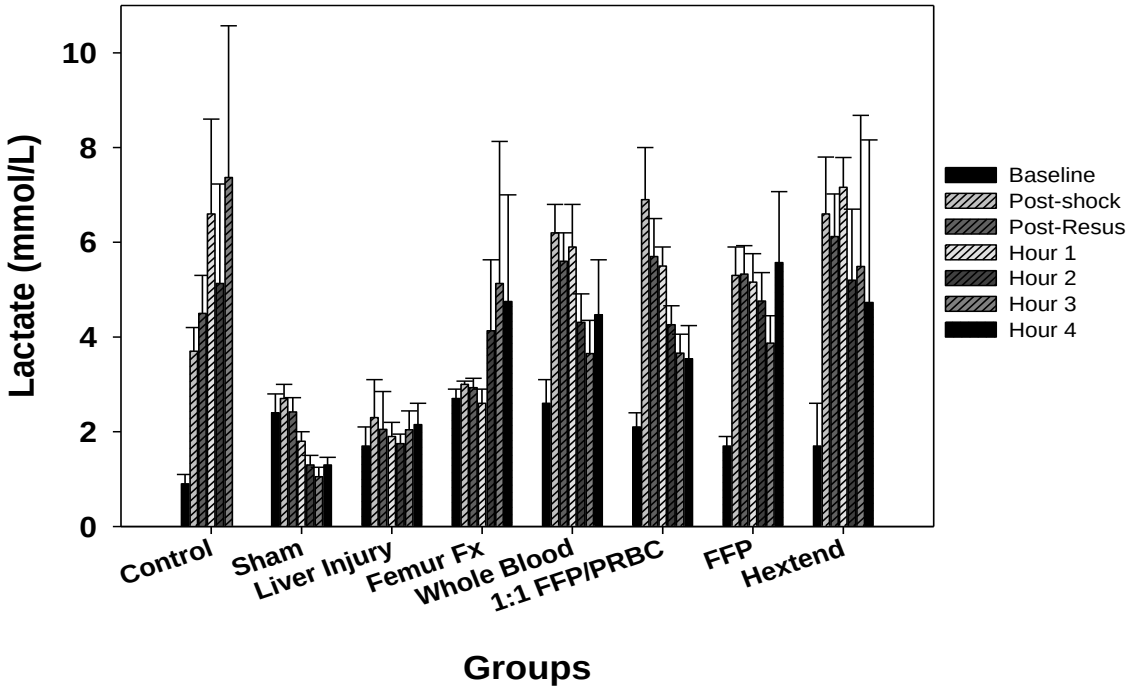


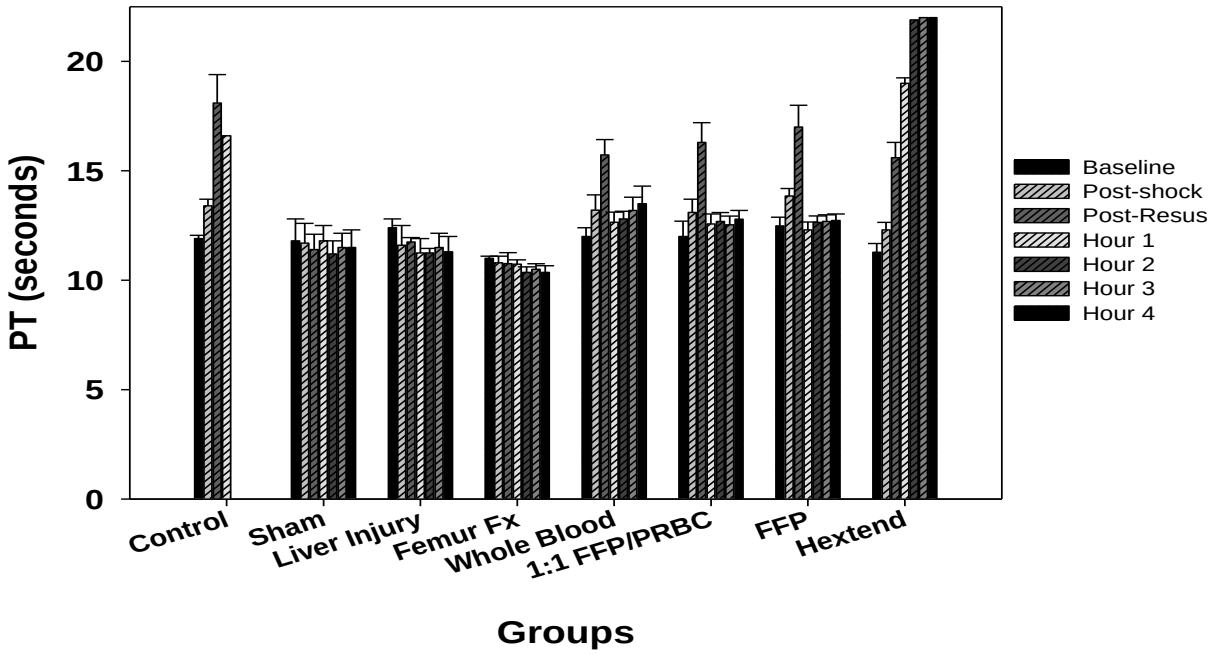
Figure 5. Lactate levels.



Coagulation parameters

Prothrombin times in all groups increased significantly after normal saline resuscitation confirming coagulopathy was produced by the model. Following treatment, PT values were reduced toward baseline in each of the blood transfusion groups. The PT was significantly increased after treatment with Hextend ($p < 0.01$). The standard error of the mean for these values was very small indicating excellent reproducibility between centers. (Fig. 6)

Figure 6. Prothrombin Time Values



Key Research Accomplishments

1. Use of Hextend in a severe multi-system model increases mortality compared to blood products including fresh frozen plasma in the absence of red blood cells.
2. Resuscitation with fresh whole blood and 1:1 PRBCs:FFP results in similar hemodynamic and physiologic outcomes.
3. Resuscitation with fresh whole blood, FFP and 1:1 PRBCs:FFP results in similar mortality and similar end of study coagulation parameters.

Specific Aim 5 – Lyophilized Plasma
(Part 1 – Desired Formulation)

This aim was a multi-center randomized trial at OHSU and USAISR. Thirty-two female Yorkshire crossbred swine were utilized. Animals were delivered 7-10 days prior to the experiment in order to minimize the stress of transport and subsequent potential changes in sympathetic output or inflammatory mediators. An overnight fasting period was observed with the exception of water ad libitum. All animals were ordered such that their weight at the time of the experiment was 36.4 ± 0.7 kg (mean ± SEM). No attempt was made to use a single vendor, and each center made their own arrangements for procurement of animals according to their standard sources. In addition to the 32 animals that were used for the study protocol, an additional 6 were used in model development and 28 for creation of the donor plasma/prbc's. The study model for this aim is the same as what was described in Specific Aim 4 except for the randomization treatments. For this trial animals were randomized to receive one of four replacement schemes that included: FFP, LP, 1:1 FFP:PRBCs, and 1:1 LP:PRBCs. The 1:1 groups were utilized because transfusion of FFP and PRBCs in a ration of 1:1 is the currently recommended standard in combat victims undergoing massive transfusion protocols. The volume of the treatment resuscitation was equivalent to the blood loss from the controlled hemorrhage.

Study Variables

Physiologic variables included survival, MAP, blood loss from the controlled hemorrhage, and blood loss due to the liver injury. Laboratory values include Hct, lactate, Plt, ABG, and electrolytes. Coagulation parameters include the PT, PTT, and ACT. Inflammatory markers were also analyzed utilizing serum and tissue cytokine analysis.

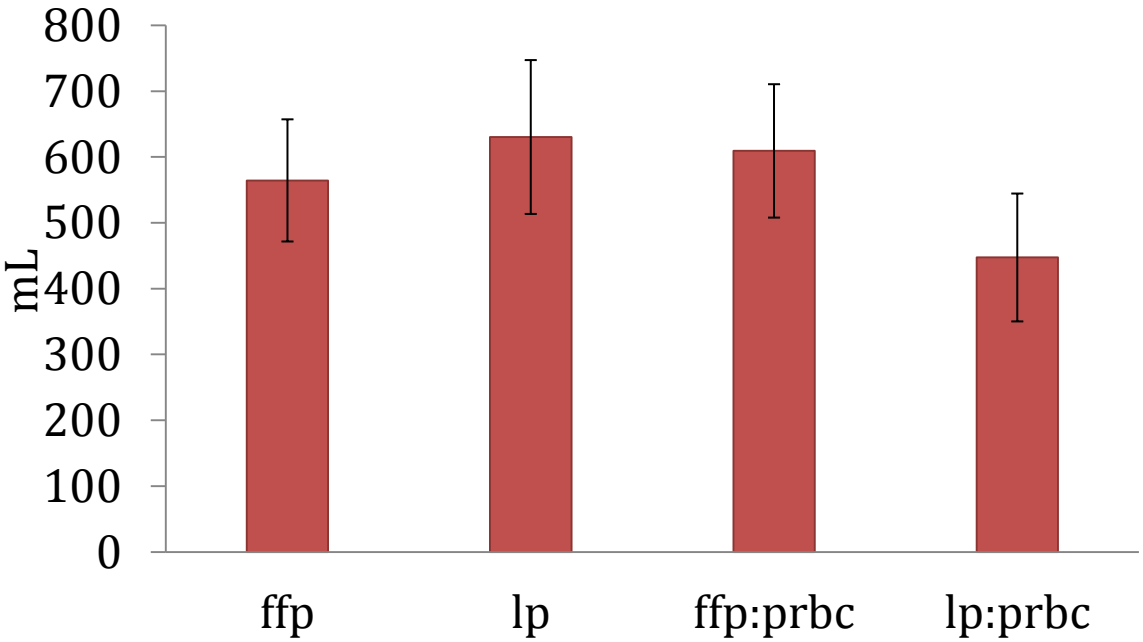
Results

Physiologic variables

Hypothermia was achieved during the shock period, with a pre liver injury temperature of 32.9 ±0.06^{o}C. Blood loss from the controlled hemorrhage, a function of the calculated blood volume, was 1602.7 ±23.5 ml or 44.3 ± 0.3 ml/kg body weight.

No significant differences were seen between the groups with respect to blood loss following liver injury. (Figure 1)

Figure 1. Post Liver Injury Blood Loss



Prior to randomization animals in all injury groups had a similar physiologic profile with an acute drop in blood pressure, followed by auto-resuscitation. Post injury mean arterial pressures (MAP) and Heart Rates across groups are shown in Figure 2 and 3. Neither MAP nor HR differed significantly between animals in the injury groups at the end of the study. Standard error bars reveal minimal variation between animals within groups.

Figure 2. Post Injury Mean Arterial Blood Pressures across Groups

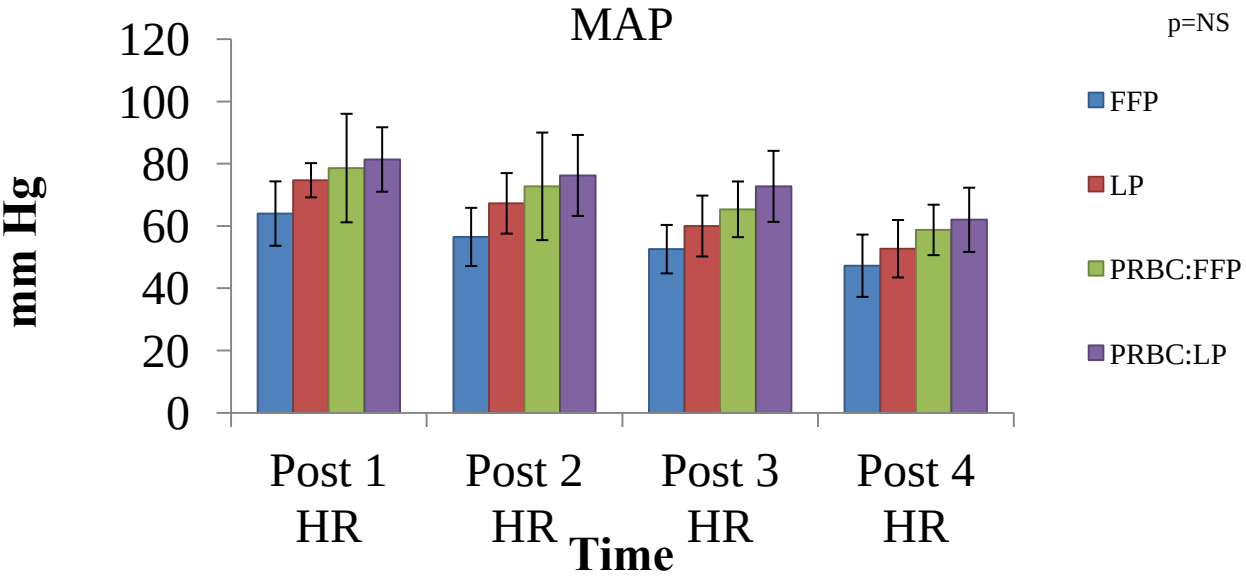
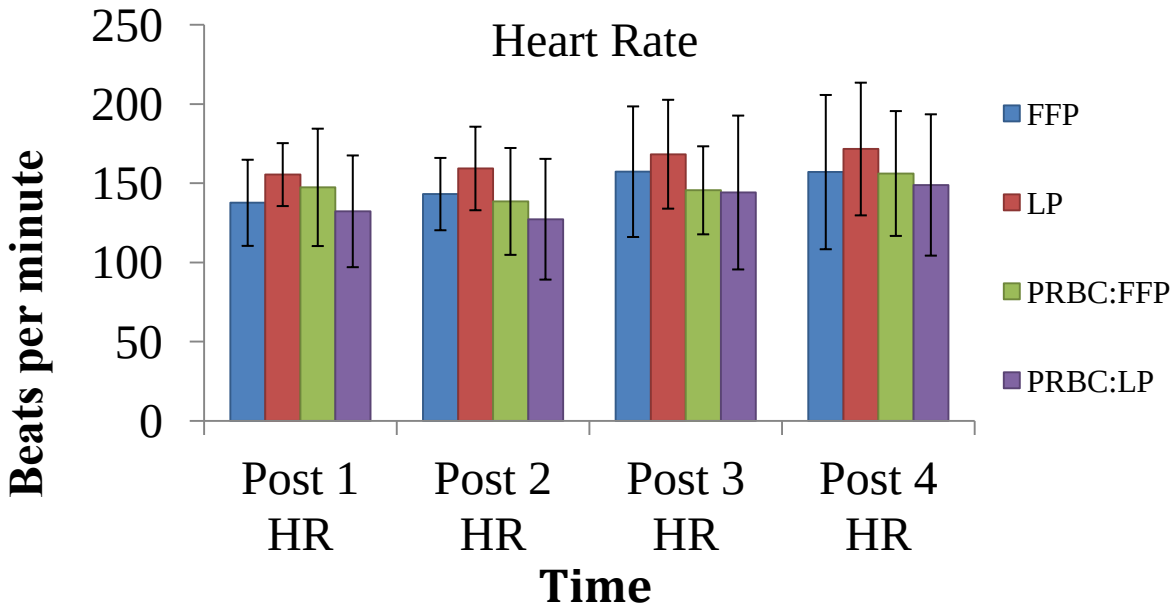


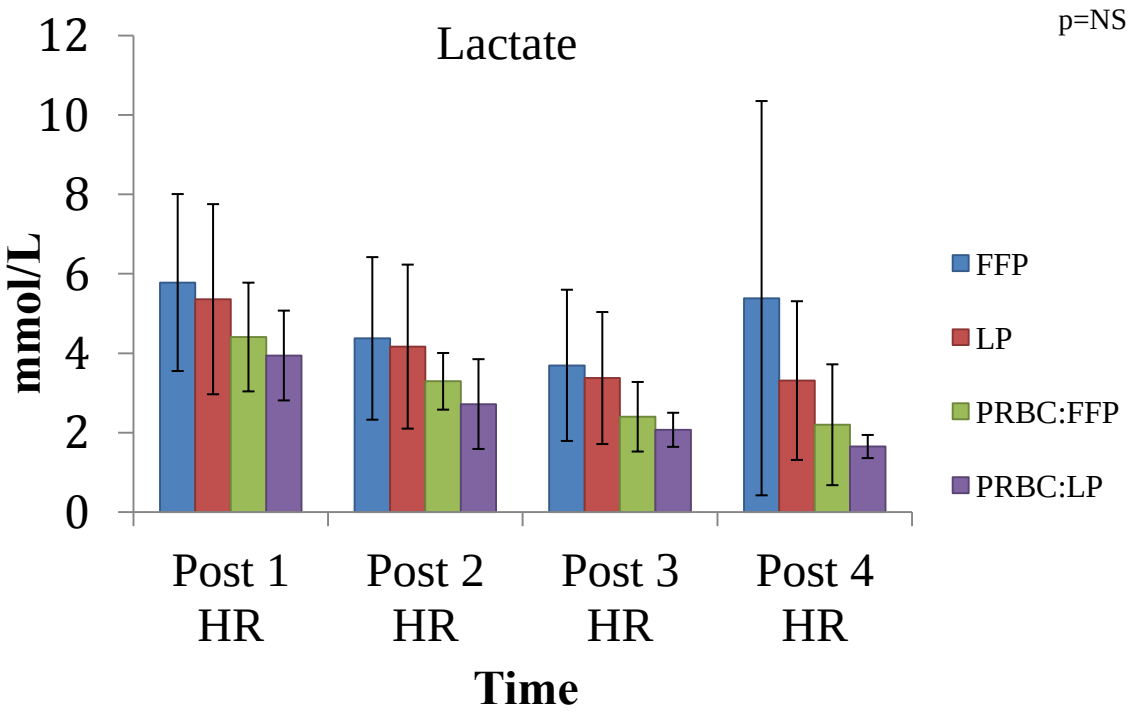
Figure 3. Post Injury Heart Rate across Groups



Laboratory variables

No statistical difference was noted between any of the groups with respect to the laboratory values (lactate, platelets, ABG, and electrolytes) that were collected. Figure 4 shows a similar progressive improvement in lactate levels among all groups with the exception of FFP.

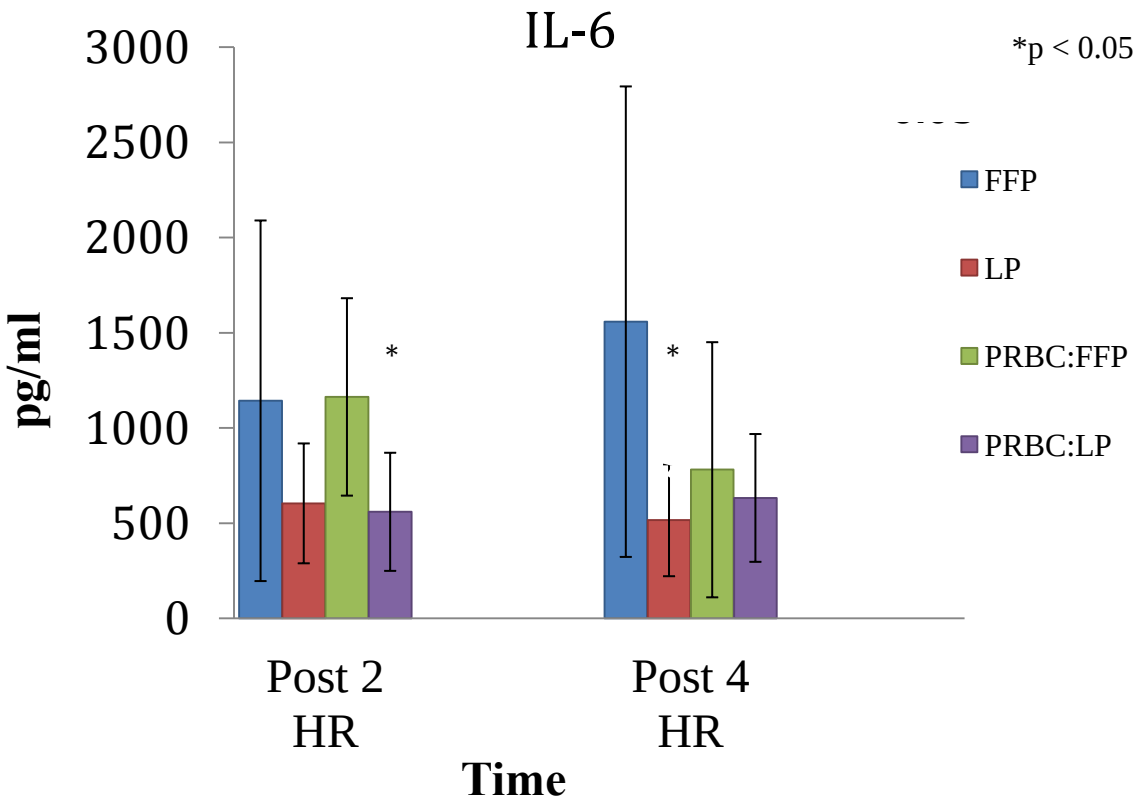
Figure 4. Post Injury Lactate Levels amongst Groups



Coagulation parameters
Activated Clotting Time, PT and PTT values were measured in all four groups at baseline and hourly for four hours after study fluid administration. Importantly, there were no differences between LP and FFP or between the 1:1 ratio groups with respect to coagulation measurements at any time.

Inflammatory Parameters
Serum samples were analyzed for markers of inflammation that included IL-6, IL-8, and TNF-α. Prior to injury, there were no differences seen between groups. Swine randomized to the FFP group had significant increases in IL-6 at both 2 and 4 hours post injury in comparison to their baseline values. In addition, TNF-α in the FFP group showed a significant increase at the 4 hour mark. A significant decrease in IL-8 production was found at the 4 hour mark in the FFP group. In swine resuscitated with 1:1 FFP:PRBC; IL-6, IL-8 and TNF-α were increased at 2h, but only TNF-α remained elevated at 4h. 1:1 LP:PRBC resuscitated swine had increased IL-8 at 2h only. In regards to the LP samples, no significant differences were seen in either IL-8 or TNF- α when compared to baseline. Compared to FFP, IL-6 levels in the LP group were significantly less at the 4 hour mark (Figure 5). Overall, LP resuscitated pigs had equivalent or less inflammation than FFP pigs.

Figure 5. IL-6 samples by group.



Key Research Accomplishments:

1. A severe multi-system combat relevant shock model can be reproduced at multiple centers.
2. The clotting activity of lyophilized plasma is favorable in comparison to FFP.
3. Resuscitation with LP results in similar hemodynamic and physiologic outcomes in comparison to FFP.
4. Resuscitation with LP, FFP and 1:1 PRBCs:FFP and 1:1 PRBCs:LP results in similar mortality and similar end of study coagulation parameters.
5. Resuscitation with LP results in less expression of IL-6 compared to FFP or 1:1 ratios at 2 HR post injury.
6. Lyophilized plasma may be superior to FFP for the resuscitation of massive trauma based on superior logistical requirements, superior retention of clotting factor function and decreased dysfunctional inflammation.

Reportable Outcomes

This work was presented and won the 2008 Region X Residents' Basic Science Competition of the American College of Surgeons Committee on Trauma.

The physiologic and coagulation components of this were presented at the Pacific Coast Surgical Association meeting in February 2009.

The inflammatory component of this work was presented at the 2009 combined meeting of the Association of Academic Surgeons and the Society of University Surgeons.

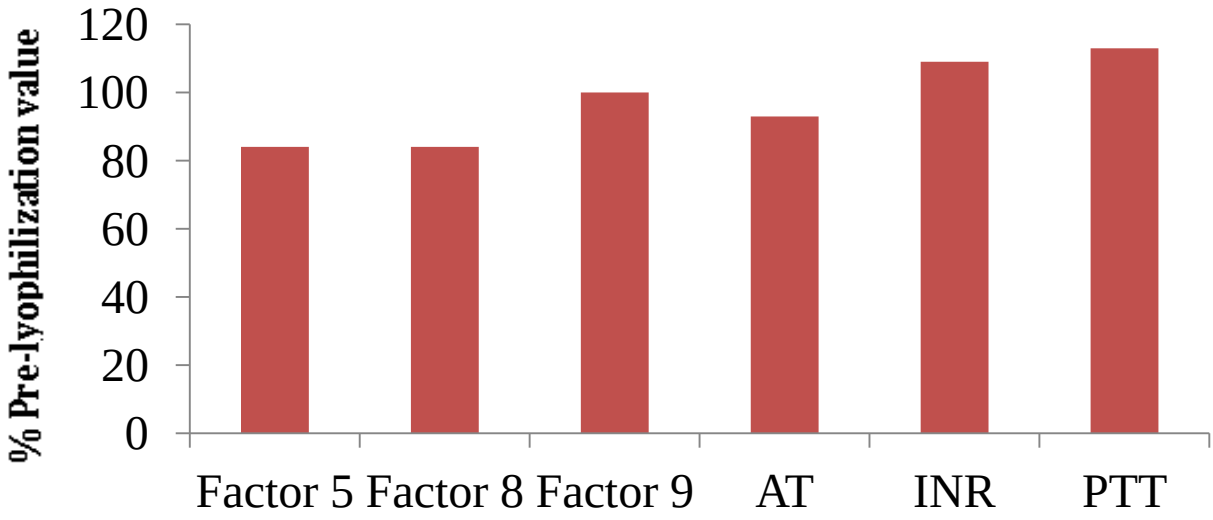
Specific Aim 5 (Part 2) – Determination of the Effect of Lyophilization on Coagulation Factor Activity

Yorkshire crossbred donor swine were anesthetized and underwent blood typing. The left ventral cervical area was sterilely prepped and the left external jugular vein was cannulated. Animals were then exsanguinated utilizing a centrifugal pump. Blood was collected in citrated Terumo Teruflex triple blood donation bags, centrifuged at 5000g for 9 minutes at 4 degrees centigrade. Plasma was removed using a Baxter Plasma Extractor. Plasma was frozen and stored at -20 degrees centigrade until collected by HemCon for creation of the lyophilized product.

RESULTS

In Vitro Analysis
Factor levels and clotting parameters were measured before lyophilization and after reconstitution. Compared to the plasma before lyophilization, there was a drop in the clotting activity by an average of 14%. This compares favorably with fresh frozen human plasma that retains only 60-70% of its original clotting factor activity. A comparison of the percent of the pre-lyophilization factor values (for industry recognized standards) is shown in Figure 1. Also included in Figure 1 is percent increase in INR and PTT compared to pre-lyophilization values.

Figure 1. Residual Clotting Activity



Specific Aim 5 (Part 3) – Determination of the Best Neutralizing Acid for Reconstitution of Lyophilized Plasma in a Multisystem Trauma Swine Model

Thirty juvenile, female Yorkshire crossbred swine were utilized and subjected to a multisystem trauma model (previously reported). In addition to the 30 animals that were used for the study protocol, an additional 12 were used in model development and 44 for creation of the donor plasma. Three animals died prior to randomization and were replaced. Donor plasma was collected utilizing a sterile procedure and the following protocol. After induction of anesthesia, initiation of mechanical ventilation and sterile preparation a cervical cutdown was performed and the common carotid artery was cannulated with an 8 French introducer (Argon Medical Devices, Athens, TX). The animals were then exsanguinated and blood was collected in citrated triple blood donation bags (Teruflex; Terumo Medical Corp, Tokyo, Japan). The collected whole blood was centrifuged at 5000g for nine minutes at 4°C and a plasma extractor (Baxter Healthcare, Deerfield, IL) was used to press the plasma into the second blood donation bag. Plasma was stored at -20°C prior to transport to a laboratory (HemCon Medical Technologies Inc, Portland, OR) for lyophilization. Sterile LP was returned and stored at room temperature for up to one month. Immediately prior to use, LP was reconstituted to its original volume with a solution of sterile water containing hydrochloric, citric or ascorbic acid for pH normalization.

Study Variables

Physiologic variables included survival, MAP, blood loss from the controlled hemorrhage, and blood loss due to the liver injury. Laboratory values include Hct, lactate, Plt, ABG, and electrolytes. Coagulation parameters include the PT, PTT, ACT, and Factors II, V, VII, VIII, IX, X, XI, XII, Protein C, Protein S.

Serum and Tissue Cytokine Analysis

Serum pro-inflammatory cytokine markers IL-6, IL-8 and TNF alpha were quantified using the Quantikine® enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN). Lung tissue was collected and rt-pcr was analyzed using Taqman Tamra Primer and Probes (Applied Biosystems, Chicago, IL). Oxidative stress was quantified using an Antioxidant Assays kit (Cayman Chemical Company, Ann Arbor, MI). Lipid peroxidation was monitored via Thiobarbituric acid reactive substance (TBARS) / malondialdehyde (MDA) assays (Cayman Chemical Company, Ann Arbor, MI). DNA damage was quantified via an ELISA Kit (Assay Designs, Ann Arbor, MI).

Results

Physiologic variables

Analysis of physiologic parameters between the three-randomization fluids showed no statistically significant difference amongst them at baseline. Baseline physiological parameters analyzed included mean arterial pressure, heart rate, temperature, and weight. Study variables included mean arterial pressure, heart rate, urine output, post-injury blood loss and survival. Mean arterial pressure was significantly less in the HCL group compared to the ascorbic group at both one and two hours ($p = .016$ and $p = .030$ respectively). MAP in the HCL group was also significantly less at the two and three hour time points ($p = .009$ and $p = .036$ respectively) in comparison to the citric group (Figure 1). There were no statistical differences in heart rate between fluids, post injury blood loss or urine output.

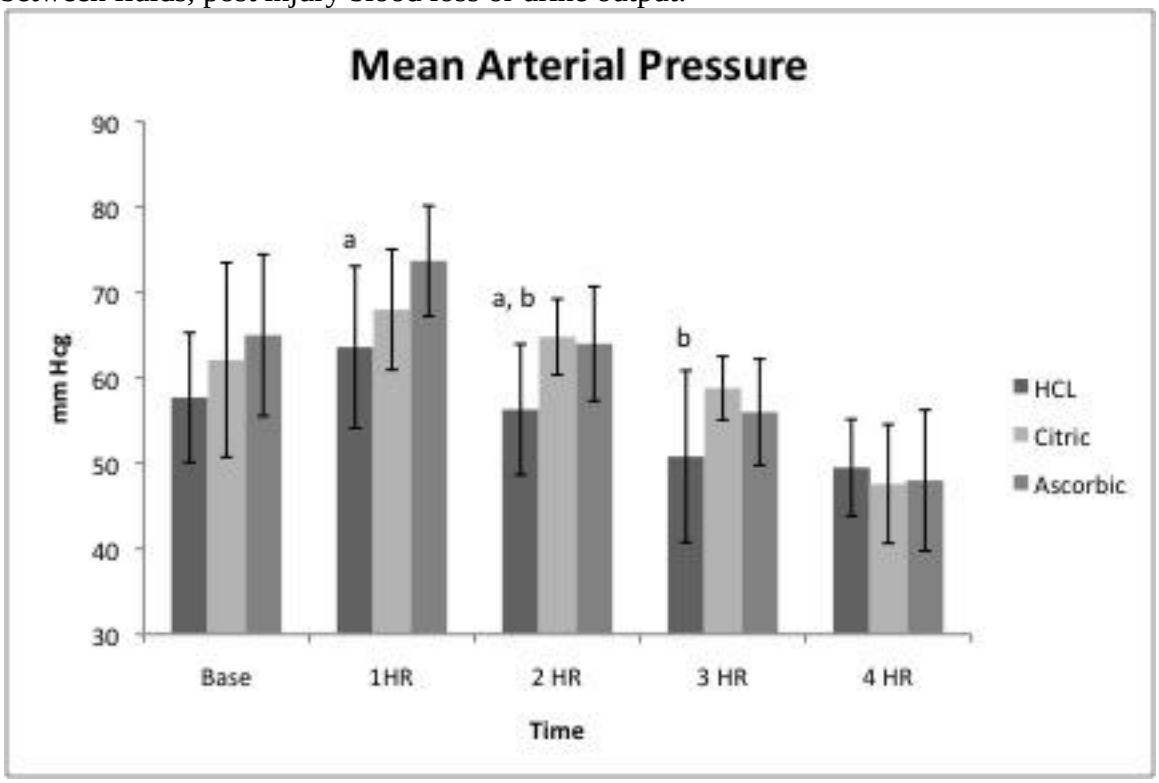


Figure 1. Mean Arterial Pressure

Laboratory value
No statistical differences were denoted between groups with respect to hematocrit, lactate, platelets, ABG or electrolytes.

Coagulation parameters
No statistical differences in the activated clotting time were seen between fluids (Figure 12). Thrombelastograms (TEG) were measured at baseline, pre-injury, and each of the four hours post liver injury. No significant differences were denoted in any TEG variables between any of the fluid groups.

Inflammatory parameters
Serum samples were analyzed for inflammatory markers including IL-6, IL-8 and TNF- α. No differences were seen at baseline or prior to injury amongst the groups. At both two and four hours post-injury, animals that received ascorbic acid expressed less IL-6 ($p = 0.03$ and 0.04 respectively) than animals that received HCL. The two-hour expression of TNF- α was significantly less in the ascorbic group ($p = 0.02$) versus the citric group. Differences within groups were denoted at multiple time comparisons for all three fluids. The HCL group yielded a higher expression of IL-6 compared to baseline at two hours ($p < 0.01$) and at four hours ($p = 0.003$). In addition, IL-6 levels were significantly higher at the four-hour time point compared to two hours ($p = 0.037$). The citric group also yielded a higher expression of IL-6 versus baseline at two-hours ($p < 0.01$) and four-hours ($p = 0.015$). The same findings were denoted in the ascorbic group as IL-6 levels were higher at two and 4 hours compared to baseline ($p < 0.01$) (Figure 2).

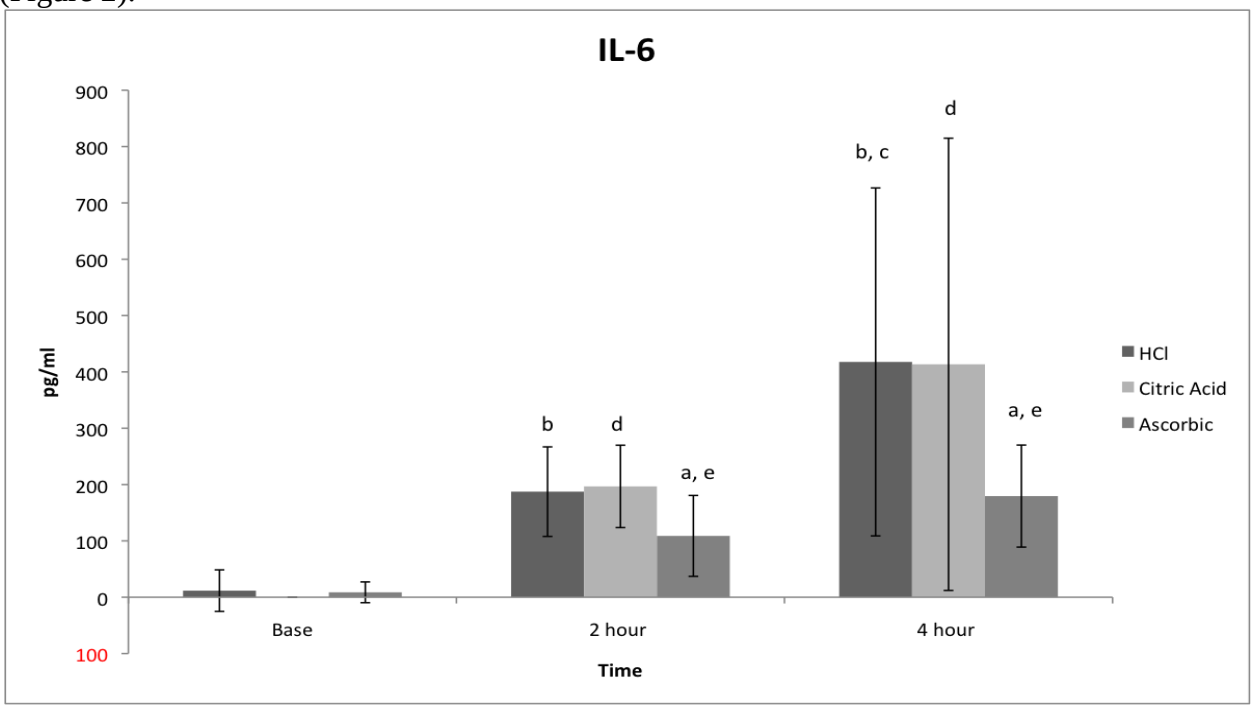


Figure 2. Expression of IL-6

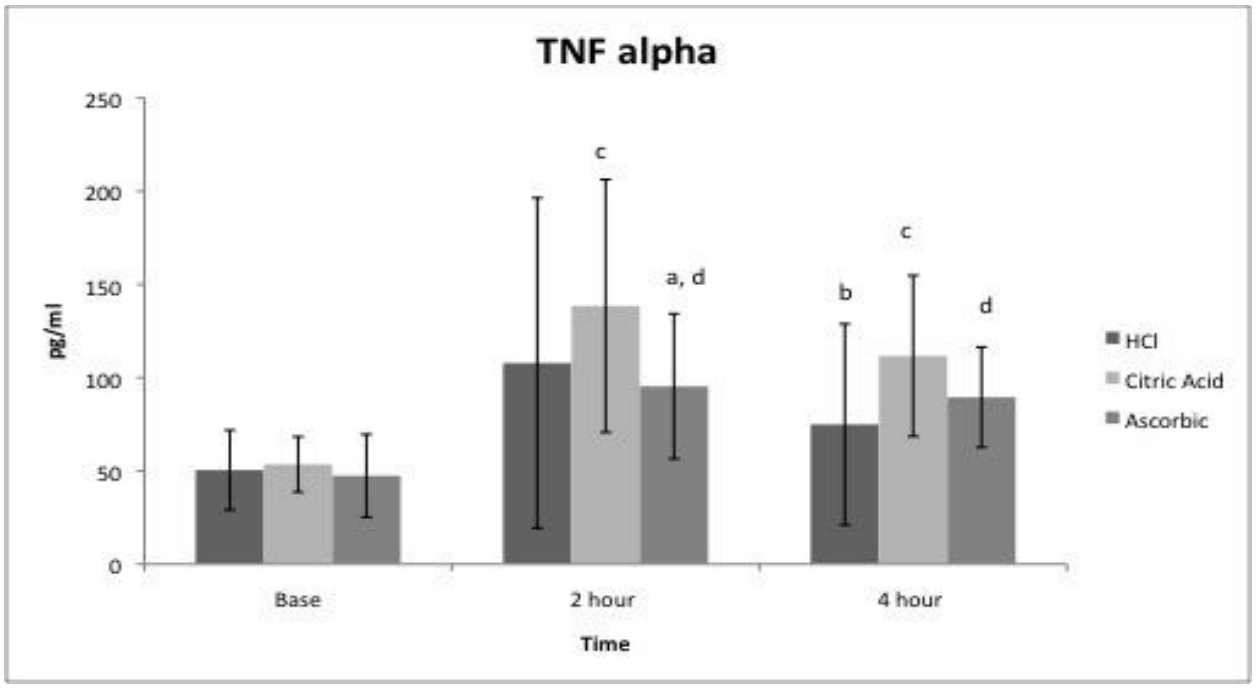


Figure 3. Expression of TNF- α

TNF- α in the HCL group was significantly less (p = 0.03) at the four-hour time point compared to two-hours. TNF- α in the citric group was significantly increased at both two-hours (p = 0.005) and four-hours (p = 0.002) compared to baseline. This was also true in the ascorbic group. (p = .001 respectively) (Figure 3). No differences were noted in the IL-8 values. No statistical differences were detected in additional assays that were analyzed that included antioxidant capacity, TBARS / MDA or DNA damage (Figure s 4-6). All three of assays elicited a trend toward increased anti-oxidant activity in the ascorbic acid group.

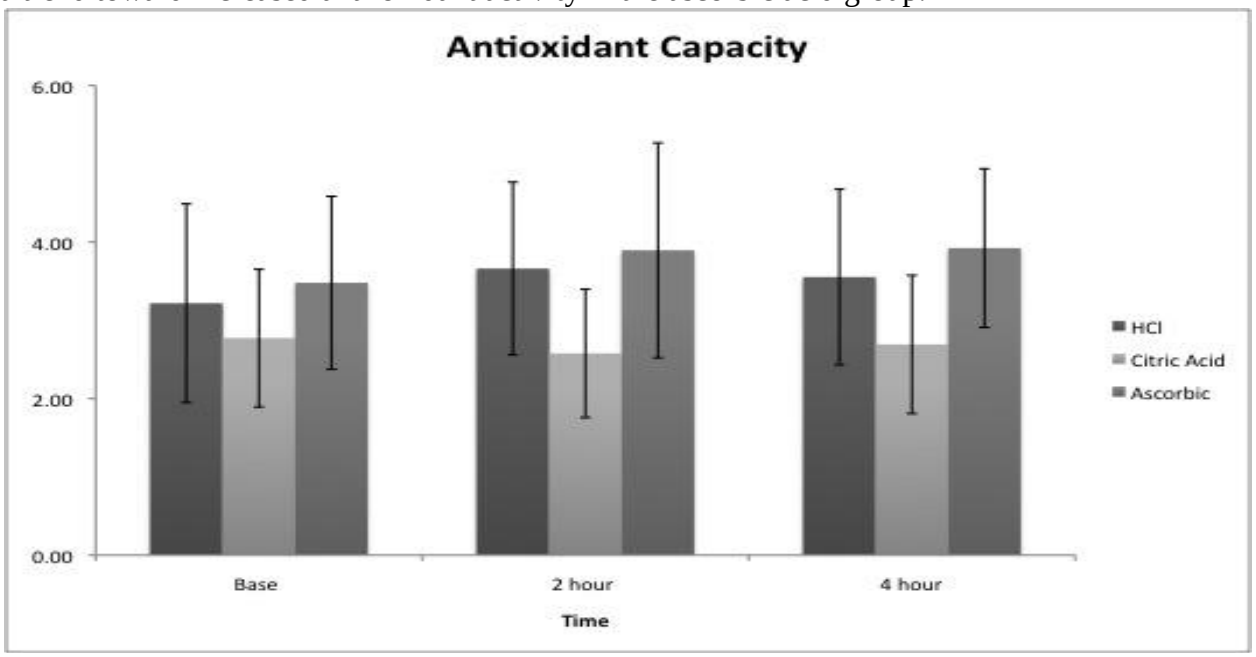


Figure 4. Antioxidant Capacity

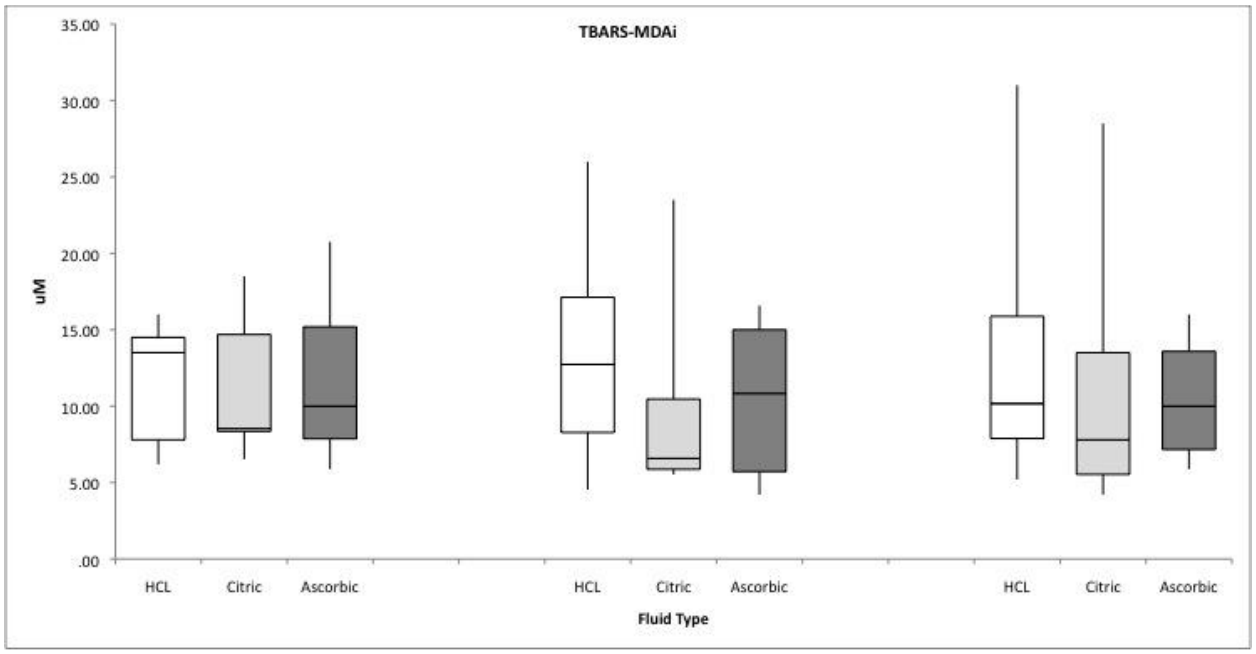


Figure 5. TBARS – MDA

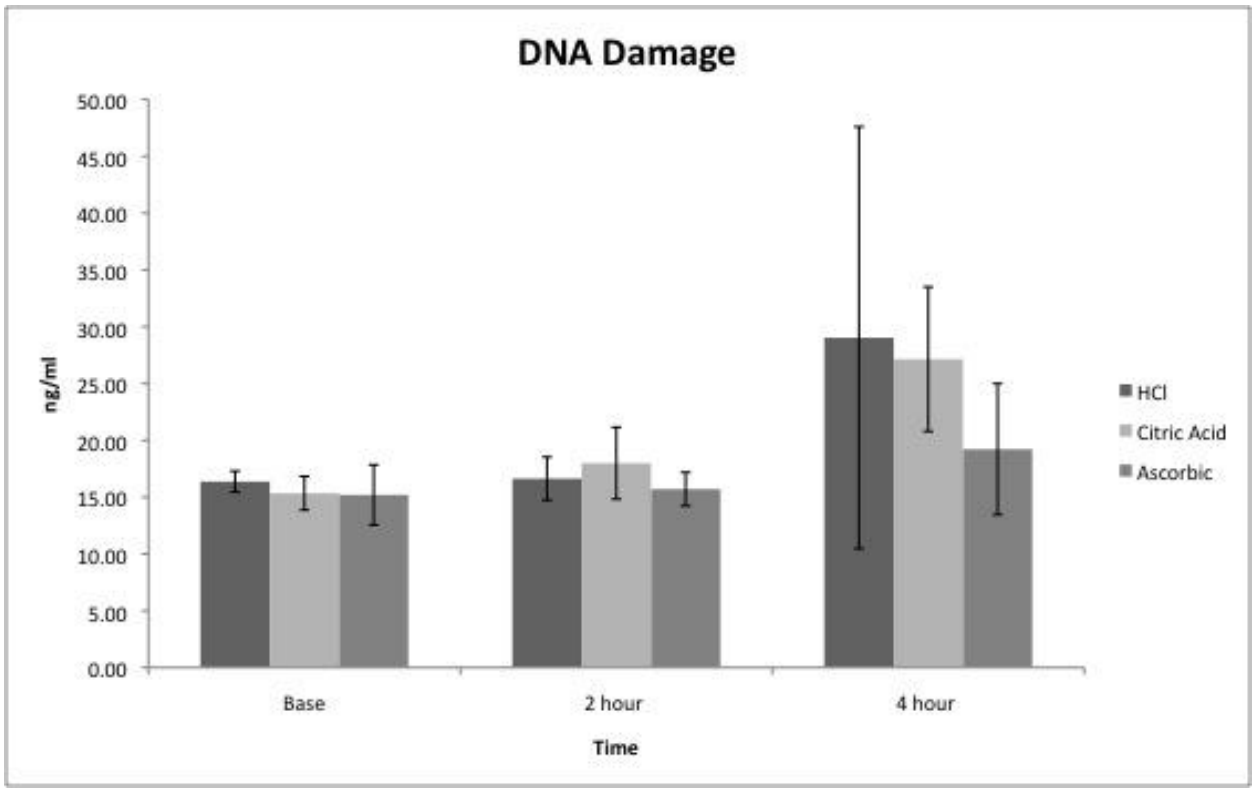


Figure 6. DNA Damage

Key Research Accomplishments:

1. There were no statistical differences between the acids with respect to physiologic parameters at baseline, however, mean arterial pressure in the HCL acid group was significantly lower than the citrate group and the ascorbic acid groups post injury.
2. There were no statistical differences seen in laboratory values between the groups.
3. There were no differences in coagulation parameters between groups.
4. Resuscitation with ascorbic acid resulted in less expression of IL-6 compared to HCL.
5. Results of the antioxidant, TBARS and DNA damage assays showed trends toward greater anti-oxidant capacity in the ascorbic group compared to either HCL or citrate.

Reportable Outcomes

This data was submitted to and presented at the American Association for the Surgery of Trauma and the Region X Committee on Trauma Competition

Specific Aim 6 – Advanced Resuscitation Strategies

INTRODUCTION:

Exsanguination is the leading cause of death on the battlefield. Lifesaving interventions include arresting hemorrhage and initiating resuscitation. The ideal resuscitation of combat casualties has not been determined. The traditional goal of resuscitation has been to restore perfusion. There is increasing evidence to suggest that early correction of coagulopathy improves mortality. Transfusion of a 1:1 ratio of plasma to packed red blood cells has been shown to result in improved survival compared to lower ratios. However, due to the need to freeze plasma and its limited availability in austere settings, it is difficult to achieve these ratios in combat scenarios. During this past year we addressed two more of the original eight specific aims from the scope of work.

In order to address specific aim 6, we attempted to determine if vasoreactive metabolites play a role in uncontrolled hemorrhagic shock. These experiments were designed to determine if novel agents that alter the affects of these metabolites could be used as adjuncts to resuscitation. Nitric oxide and the P450 eicosanoids epoxyeicosatrienoic acids (EETs) play a major role in vasoreactivity and inflammatory states following trauma. EETs are primarily produced by vascular endothelium and they serve as major components of key vasoregulatory mechanisms. EETS play an important role in regulating tissue perfusion in the heart, brain and kidney. The natural history of EETS production during hemorrhagic shock with and without resuscitation has not been studied nor have the effects of blocking and potentiating these important vascular mediators.

Materials and Methods (Part 1)

The Effect of Uncontrolled Hemorrhagic Shock on Metabolic Parameters in an Uncontrolled Hemorrhagic Shock Swine Model

The model was developed at the Oregon Health & Science University (OHSU), and approved by the Institutional Animal Care and Use Committee. This model is known to produce a rapid decrease in mean arterial blood pressure followed by a spontaneous increase in blood pressure (auto-resuscitation) after bleeding subsides.

A femoral artery cut down was performed to place a 4-F aortic catheter with an integrated thermistor tip (Pulsion Medical Systems, Munich, Germany) for continuous hemodynamic parameters using PiCCO-Technology that was connected to the PiCCO *plus* monitor (Pulsion Medical System, Munich, Germany). The PiCCO technology system allows hemodynamic monitoring through two different techniques, either intermittently by transpulmonary thermodilution or continuously by pulse contour wave analysis. It is a validated, less invasive alternative to the Swan-Ganz catheter for the measurement of cardiac output (CO). For transpulmonary thermodilution, a bolus (10 ml per bolus) of cooled (0-6°C) crystalloid fluid was injected through a venous catheter and the thermistor tipped arterial catheter placed through the femoral artery would measure the subsequent temperature changes. These measurements were done manually and randomly throughout the respiratory cycle to obtain CO and stroke volume SV measurements and calculated systemic vascular resistance values. Thermodilution is also used for calibration of the pulse contour method for continuous measurements of SV and CO.

The Sievers® Nitric Oxide Analyzer NOA 280i (GE Analytical Instruments, Boulder, CO) was utilized for the detection of Nitric Oxide (NO) metabolites. Employing ozone-chemiluminescence technology, the NOA 280i has unsurpassed versatility for liquid NO measurement. The NOA 280i measures nitric oxide, nitrite, nitrate or nitrosothiols in virtually any biological fluid.
Plasma samples were collected for analysis of eicosanoids. Primary analysis focused on Epoxyeicosatrienoic acids (EETs) and other P450 metabolites by liquid chromatography mass spectrometry with a reagent from Cayman Chemical (Ann Arbor, Michigan).

Sixteen animals were utilized for this experiment. In addition to the study animals, an additional ten were used for model development. Prior to injury, two animals died from heart related issues and were replaced. Animals were fasted for 16 hours the day before surgery. Water was available ad libitum. On the day of the experiment animals were given an induction agent consisting of TelazolR 8 mg/kg given intramuscularly. Animals were placed in the supine position. A 7.5mm internal diameter cuffed endotracheal tube was placed. The endotracheal tube was connected to the anesthesia machine with 1-3% isoflurane for anesthetic maintenance in 50% oxygen. Tidal volume was fixed at 10 ml/kg with a rate of 10 breaths per minute. An esophageal stethoscope, gastric tube and thermometer was inserted. An EKG monitor was secured and continuous monitoring started. A left ventral cervical cutdown was made and an 8F polyethylene catheter was placed in the carotid artery and the external jugular vein. The cervical arterial catheter was used for continuous blood pressure analysis and blood sampling, while the venous catheter was used for administration of study fluid. A right ventral cervical cutdown was made and a cordis introducer was placed in the right external jugular vein. A Swann-Ganz catheter was floated through this introducer. A femoral cutdown was made and a PICCO catheter placed in the femoral artery for PICCO measurements.

Baseline electrolyte panel, ionized calcium, prothrombin time, partial thromboplastin time, fibrinogen, thrombin-antithrombin complexes, nitric oxide and EETs samples were drawn. A ventral midline incision was made and a laparotomy performed. A cystostomy completed and a Foley catheter placed. The abdominal wall was closed with towel clips.

The animals were allowed to stabilize for a minimum of 15 minutes. During this time period temperatures were standardized to 38ºC.

After exposing the femoral artery and vein, animals were randomized to either sham or hemorrhage (HEM). The sham animals received no injury and were monitored for two-hours while Metzenbaum scissors were used to transect both vessels in the hem group. Blood loss was collected via suction. The suction canister was placed on a scale capable of real-time continuous weight recording. Once the blood loss volume was equivalent to 30% of the animal's total blood volume (BV (cc/kg) = 161.475 x wt (kg) $^{-0.2197}$), the bleeding vessels were clamped and ligated to achieve hemostasis.

Animals were monitored for 2 hours post injury with hemodynamic variables monitored continuously. Labs evaluating nitric oxide and EET's variables were collected at 5, 10, 20, 30, 60 and 120 minutes post injury. Arterial blood gases were evaluated at 60 and 120 minutes.

Following completion of the 120 minutes of post injury follow-up, surviving animals were euthanized and analysis of the injury conducted to verify a standard injury.

Study Variables

Physiologic variables included survival, MAP, HR, PICCO, cardiac output, urine output, blood loss from the controlled hemorrhage. Laboratory values include Hct, lactate, Plt, ABG, electrolytes, nitric oxide and EETs.

Results

Physiologic parameters

Analysis of physiologic parameters between the shams and HEM group showed no statistically significant difference amongst them at baseline. Physiological parameters analyzed included mean arterial pressure, heart rate, cardiac output, temperature, and weight. Mean arterial pressure was significantly less ($p < 0.01$) in the hem animals at 5, 10, 20 and 30 minutes post injury (Figure 1). The pulmonary artery pressures demonstrated similar results with significantly decreased values ($p < 0.01$) at 5, 10 and 20 minutes post injury (Figure 2). Cardiac output was significantly less in the hem group at 5 minutes post injury ($p = 0.05$). Values continued to be significantly less ($p < 0.001$) at minutes 10, 20, 30, 60 and 120. Even though the values remained significantly lower, a trend towards recovering back to baseline was noticed in the HEM group (Figure 3).

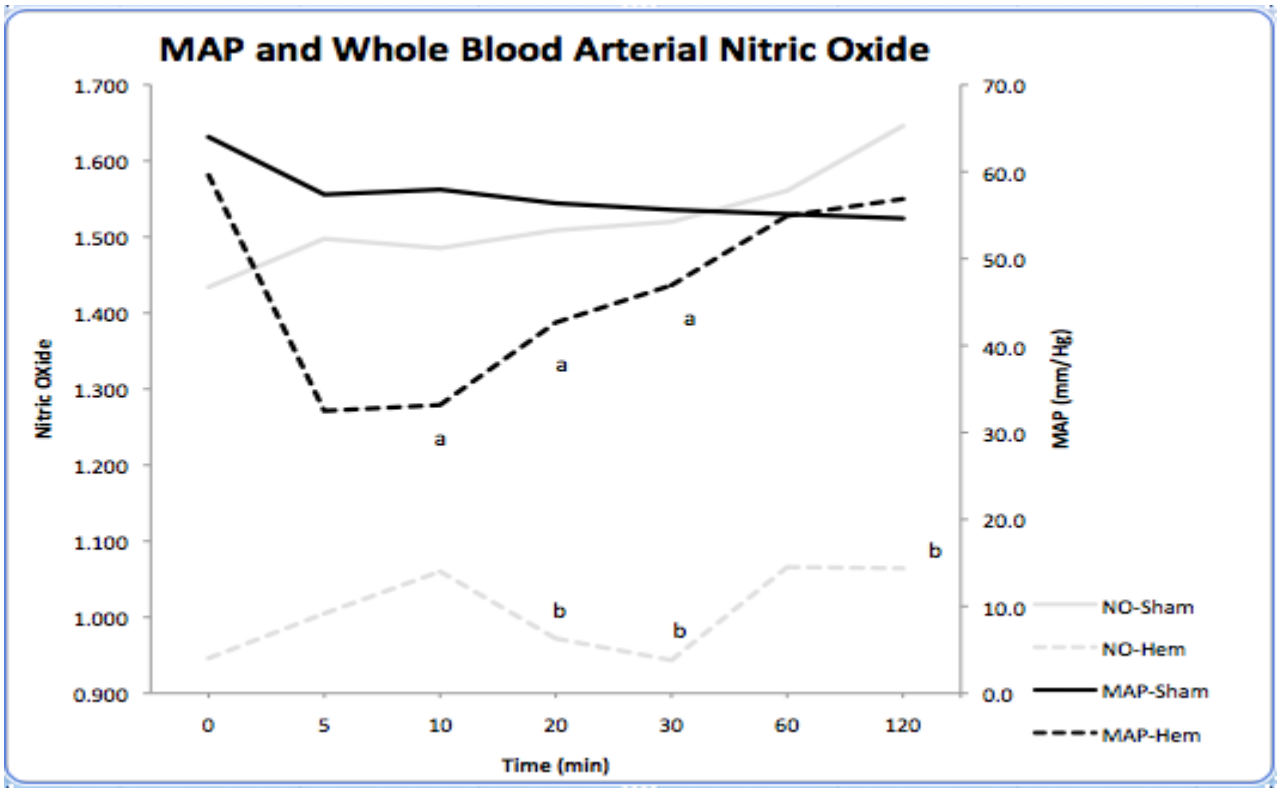


Figure 1. Mean Arterial Pressures with Nitric Oxide values

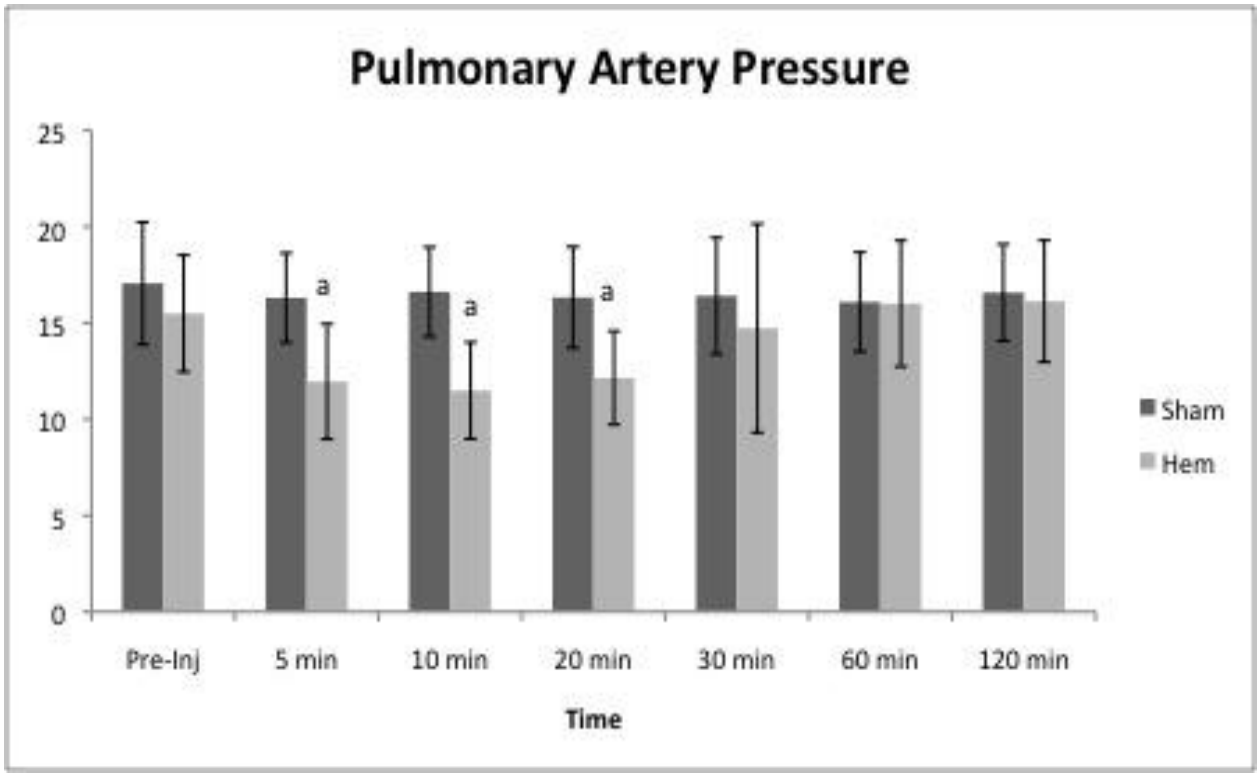


Figure 2. Pulmonary Artery Pressures

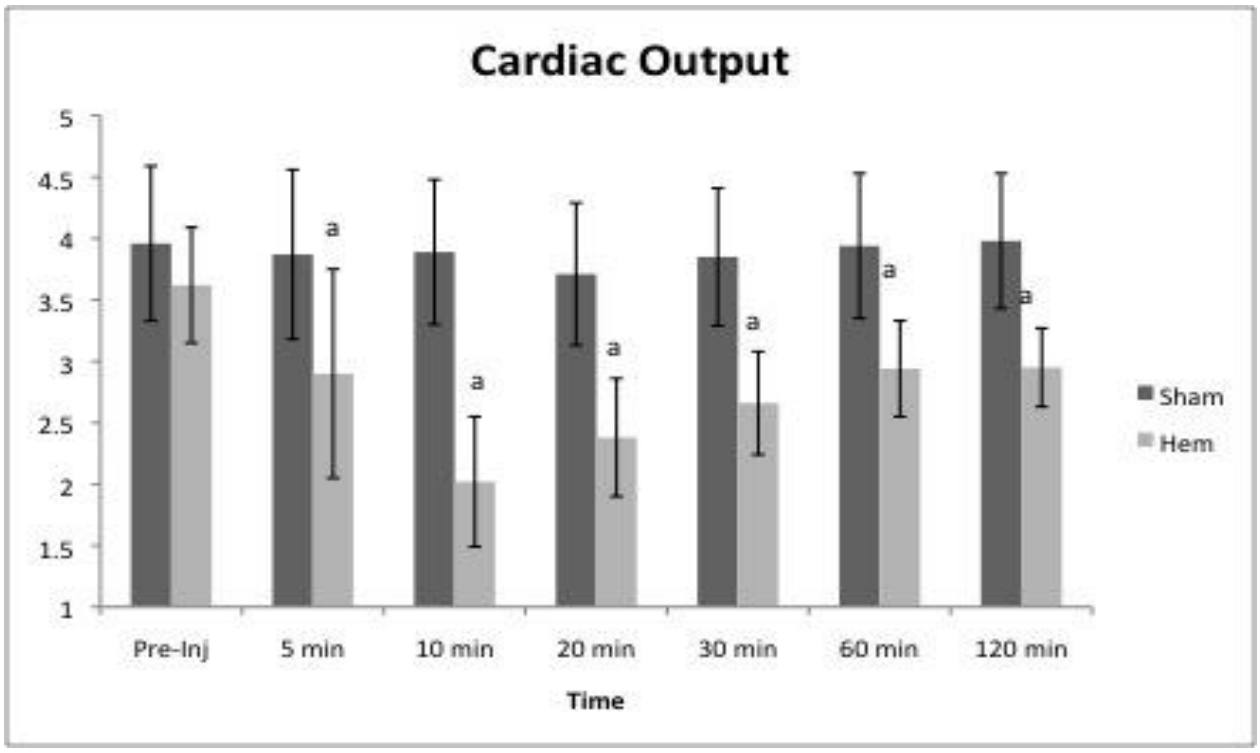


Figure 3. Cardiac Output

Laboratory parameters

No statistical differences were noted between groups with respect to lactate, platelets, ABG or electrolytes. Hematocrit values were significantly higher ($p < 0.05$) in the hem group at 30, 60 and 120 minutes post injury (Figure 4).

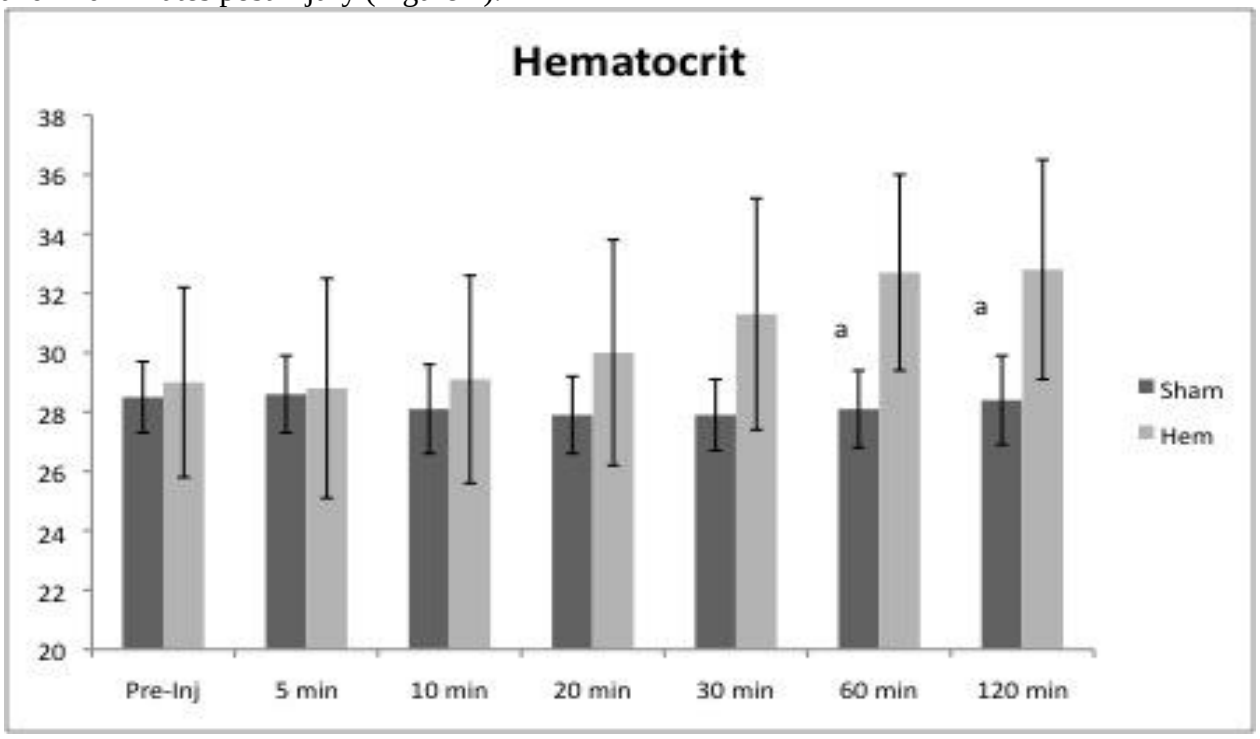


Figure 4. Hematocrit Levels Post-Injury

Nitric Oxide parameters

Analysis of parameters related to nitric oxide measurements in arterial whole blood resulted in significant differences between the sham and hem groups. No differences were noted at baseline. Following injury, sham animals elicited higher values than hemorrhage animals at 20 minutes (1.509 vs. .972 p = 0.034), 30 minutes (1.520 vs. .944 p = .027) and 120 minutes (1.646 vs. 1.064 p = .044) respectively.These differences in nitric oxide correspond with differences in mean arterial pressure and cardiac output at those times. The decrease in NO may explain the spontaneous elevation in blood pressure that occurs during the "auto-resuscitation" period. The spontaneous elevation in blood pressure may be secondary to vasoconstriction suggesting the possibility of prolonging the period of hypotensive resuscitation by modifying the NO response.

Epoxyeicosatrienoic acids (EETs)

No differences were denoted in Epoxyeicosatrienoic acids between groups.

Key Research Accomplishments

1. Moderate to severe injury without fluid resuscitation yielded similar physiological results between hemorrhage and sham animals 30 minutes post injury.
2. Un-resuscitated, uncontrolled hemorrhagic shock results in increased HCT early post-injury.
3. Acute uncontrolled hemorrhagic shock is associated with an increase in whole blood nitric oxide levels. Spontaneous elevation in blood pressure following hemorrhagic shock is associated with decreased nitric oxide levels, which may produce vasoconstriction.

Reportable Outcomes

This data was presented to Portland Surgical Society and Oregon /Washington chapter of ACS as well as to the Committee on Shock.

Materials and Methods (Part 2)

The model was developed at the Oregon Health & Science University (OHSU), and approved by the Institutional Animal Care and Use Committee.

Female Yorkshire Crossbred swine underwent the following polytrauma protocol to assess the efficacy of Tranexamic acid (TXA). This drug is commonly prescribed for control of excess bleeding. To date only a single trial in trauma (CRASH-2) has been conducted. This study does not answer the mechanistic questions in relation to controlling hemorrhage. We were hoping to identify that with this project.

Efficacy of Tranexamic Acid in a Model of Polytrauma

Nineteen swine (12 study, 6 model development, 1 death) were utilized for this experiment. Animals were delivered 7 to 10 days prior to the experiment in order to minimize the stress of transport and subsequent potential changes in sympathetic output or inflammatory mediators. Animals were fasted for 16 hours the day before surgery. Water was available ad libitum. A single vendor was used to eliminate potential differences in animal strain.

Monitoring, access and pre-experiment procedures

After swine were anesthetized a left cervical cutdown was performed and polyethylene catheters were inserted respectively into the left common carotid and left external jugular vein. The arterial line was utilized for the controlled hemorrhage and blood sampling throughout the experiment while the venous line was used for administration of bolus resuscitation fluids and TXA. Finally, a proximal femoral cutdown was performed and the artery was cannulated for continuous blood pressure monitoring. Mean arterial pressure (MAP) was continuously recorded and averaged every 10 seconds with a blood pressure analyzer and digital data collection system (DigiMed, Louisville, KY). Baseline labs were collected and included electrolytes, lactate, spun hematocrit (Hct), activated clotting time (ACT), platelets (Plt), prothrombin time (PT), partial thromboplastin time (PTT), and arterial blood gas (ABG) and additional coagulation factors. In addition, a baseline thrombelastogram (TEG, Haemoscope Corporation, Niles, IL) was performed. A celiotomy was then performed, at which time a suprapubic bladder catheter was placed to monitor urine output.

Injury Phase

After needle localization, a captive bolt gun was used to fracture the femur and create a soft tissue injury at the midshaft of the left femur. A controlled hemorrhage was then initiated to remove 50% of the blood volume based on a published, standard equation relating blood volume to body weight for domestic swine. During hemorrhage if the mean arterial blood pressure (MAP) fell below 25mm/Hg, normal saline (NS) was infused at a rate of 165 ml/min to keep the MAP>25 mm/Hg. The animal was also cooled to 33 +/-0.4^{o}C using cooled intraperitoneal lavage with crystalloid as needed (most of the animals developed a degree of hypothermia spontaneously due to shock and infusion of IV fluids). These procedures were followed by a 30-minute shock period, representing time in the field prior to medical intervention.

Prehospital care/transport phase

After the 30-minute shock period, electrolytes, spun hematocrit, ACT, PT, PTT, platelets, ABG, factors and TEG were again recorded. After coagulation studies and lab collection, the hemorrhage volume was replaced with a 3:1 ratio of NS infused at a rate of 165 ml/min, minus any given during the controlled hemorrhage. This reflects current civilian pre-hospital resuscitative practices. At the same time as the replacement fluid infusion, the TXA or the control (Normal Saline) drip is initiated at a bolus of 25 cc over 10 minutes. After the initial 10-minute bolus, a drip is continued over the next 4 hours and 26 minutes of 13.83 cc.

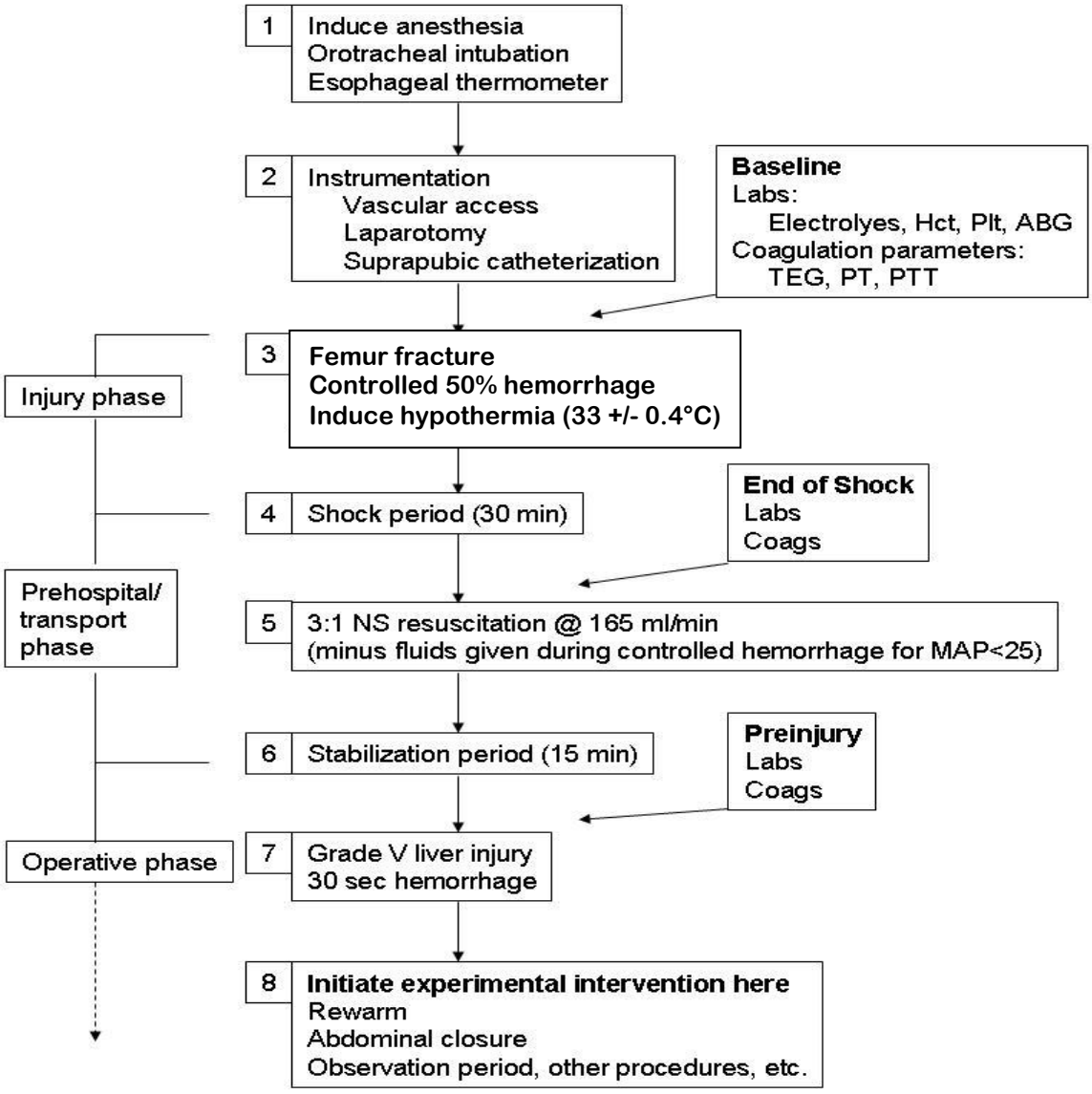
Operative phase

Following NS resuscitation, a 15-minute stabilization period was observed; during which a baseline MAP was recorded and pre-weighed laparotomy sponges were placed in both paracolic gutters and in the pelvis for blood collection. Labs and coagulation studies were again collected, and a previously described grade V liver injury was created at the confluence of the right and middle hepatic veins using a specialized clamp.
Thirty seconds of hemorrhage were then followed by evacuation of blood from the abdomen and packing of the liver with a fixed number of additional pre-weighed laparotomy sponges. The liver injury was designed to provide a second stressor after initial injury and also to create a standardized injury that had the potential to re-bleed, both of which simulate a laparotomy after trauma in a patient with solid organ injury. Thirty seconds after injury, the liver was packed with laparotomy sponges in a standardized fashion. The animal was also re-warmed to 37°C and the abdomen closed with towel clips. These animals received no additional fluid replacement after the liver injury.

Follow-up

Animals were monitored for 4 hours post injury or to death. Labs were collected at 1, 2, 3 and 4 hours. If the MAP fell below 15 mmHg it was denoted as death and the time of death was recorded. Animals surviving 4 hours were euthanized with Euthasol.

Lung tissue was collected at the end of 4 hours or at declaration of death for rtPCR analysis. Tissue was stored in RNA later and a 10% buffered formalin solution. An autopsy of the liver was performed to ensure comparable injuries without portal vein involvement.



Study Variables

Physiologic variables included survival, MAP, blood loss from the controlled hemorrhage, and blood loss due to the liver injury. Laboratory values include Hct, lactate, Plt, ABG, and electrolytes. Coagulation parameters include the PT, PTT, ACT, and Factors II, V, VII, VIII, IX, X, XI, XII, Protein C, Protein S

Statistical Analysis

The study was terminated prior to completion due to premature death of all animals prior to the 4-hour time point.

Specific Aim 7 – Comparison of Inhalational Anesthetic

Part 1 - *Comparison of isoflurane and total intravenous anesthesia in uncontrolled hemorrhagic shock.*

Twenty female Yorkshire crossbred swine were randomized blindly to receive either 1-3% inhaled ISO, or IV ketamine (15-33mg/kg/hr) with midazolam (1-2mg/kg/hr), and buprenorphine (0.5-10 mcg/kg/hr) for maintenance anesthesia. Animals were sedated with Telazol (4mg/kg) and induction was performed using ISO, followed by orotracheal intubation. An aural IV was placed, and randomized maintenance anesthesia was initiated and monitored by an animal technician who was independent of the study team. Depth of anesthesia was monitored in both groups using standardized criteria such as jaw laxity and painful stimuli to the nasal septum and forefoot. Animal temperature was controlled with external warming devices. Invasive lines were placed for continuous blood pressure recording and fluid resuscitation. Tissue oxygenation was measured in the left groin using near infrared spectroscopy. Celiotomy, splenectomy, and bladder catheterization were performed. After a 15-minute stabilization period, baseline mean arterial pressure (MAP) was documented and a grade V liver injury created. This was followed by uncontrolled hemorrhage for 30 minutes. Animals were resuscitated with 8ml lactated Ringer's per ml blood loss at 165 ml/min. This volume was based on prior studies in our laboratory using the same model comparing total blood loss with total volume of fluid required to maintain the baseline MAP. The rate of infusion is half the rate administered by a Level I infuser as the animals were approximately half the weight of a normal human. MAP and tissue oxygen saturation (StO2) were continuously monitored. Laboratory data were collected every 30 minutes, and the animals were sacrificed at 120 minutes after injury.

Based on standard criteria, all animals in both groups were adequately anesthetized using the stated ranges of anesthetics. Baseline weight (31.1 $\pm$ 0.8 kg vs. 32.5 $\pm$ 0.8 kg), number of central veins injured (1.8 for TIVA vs. 1.9 for ISO, $p>0.1$) and blood loss (796 $\pm$ 70 ml for TIVA vs. 791 $\pm$ 91 ml for ISO, $p>0.1$) were similar. Two animals in each group died prior to completing the fluid resuscitation. As shown in Figure 1, the ISO group had a lower baseline MAP (76 $\pm$ 4.0 vs. 89.5 $\pm$ 3.6, $p=0.02$), lower MAP at injury (66.6 $\pm$ 4.3 vs. 86.4 $\pm$ 4.3, $p<0.01$), and lower MAP at study completion (56.7 $\pm$ 2.7 vs. 74.9 $\pm$ 3.4, $p<0.01$). Nadir blood pressures were equivalent, and there was no difference between the two groups in the ability to return to the baseline MAP. Following resuscitation, the MAP decreased in the ISO group but stayed the same in the TIVA group. StO2 values were lower for ISO at the time of injury but were similar between the two groups for the remainder of the study. The acute rise in StO2 in the ISO group at 20 minutes is the result of 2 pigs dying at nearly identical time points. The ISO group had a lower lactate (3.7 $\pm$ 0.7 vs. 6.9 $\pm$ 1.1, $p=0.04$) and higher pH (7.48 $\pm$ 0.02 vs. 7.39 $\pm$ 0.03, $p= 0.04$) thirty minutes after injury but equalized following resuscitation (Figure 2).

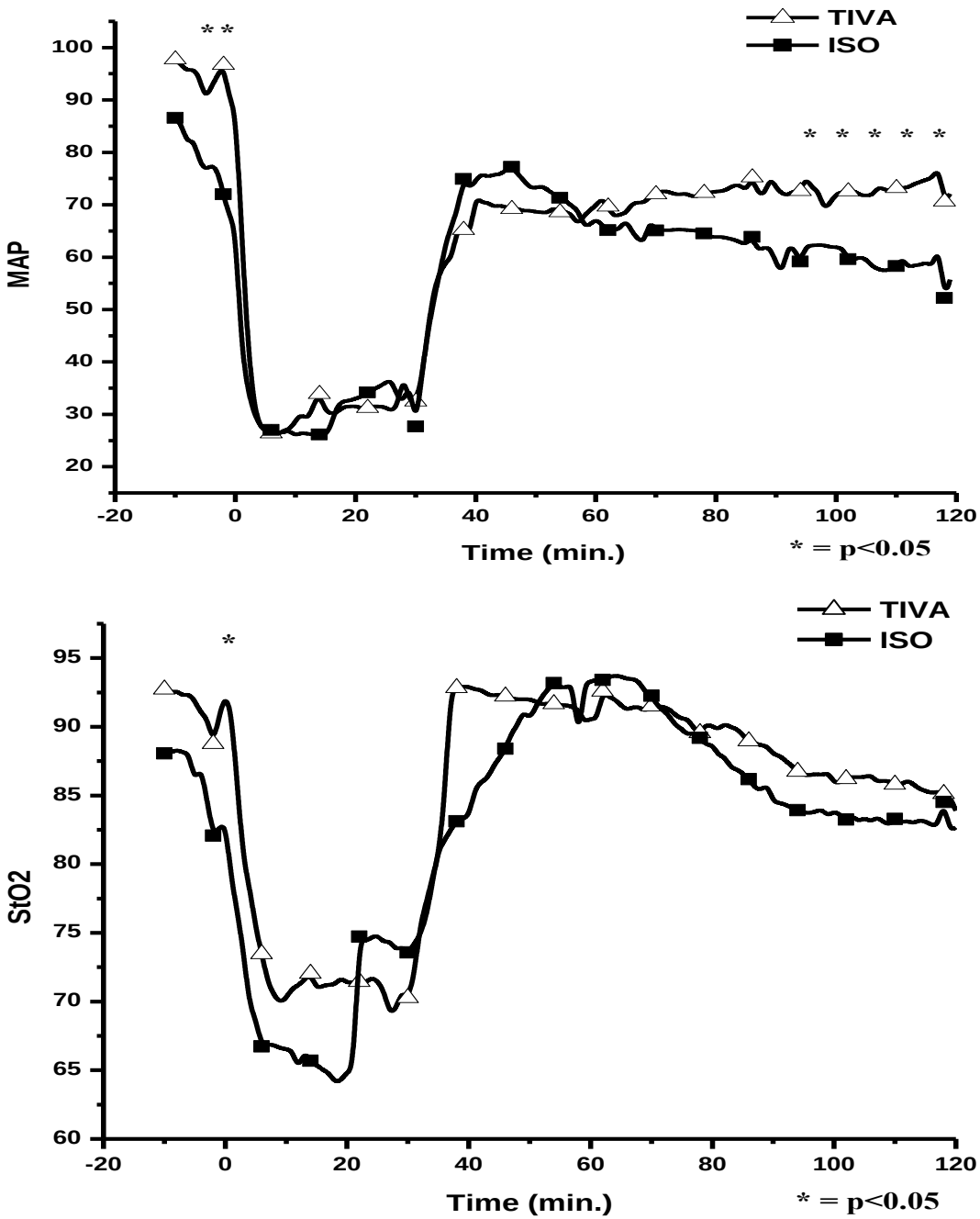


Figure 1. The effect of TIVA vs. isoflurane on MAP and StO2. Time point 0 represents injury.

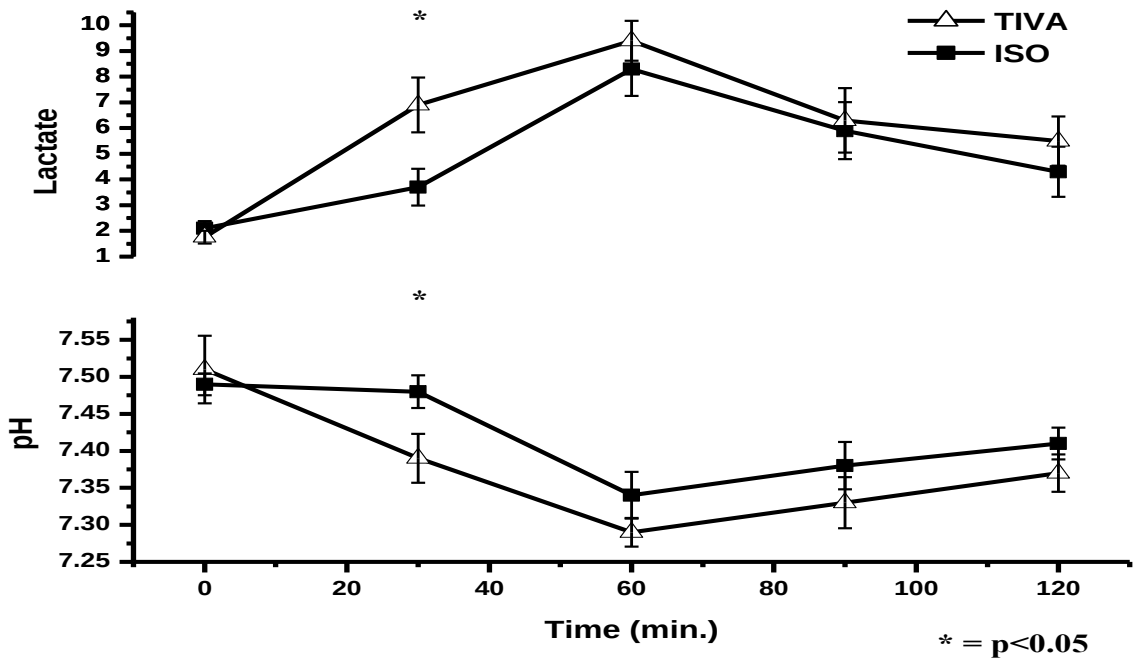


Figure 2. Comparison of pH and lactate. Time point 0 represents baseline.

Key Research Accomplishments – Part 1

1. In a swine model of uncontrolled hemorrhagic shock, total intravenous anesthesia produces adequate and comparable anesthesia to that of isoflurane.
2. Total intravenous anesthesia produces a higher MAP at baseline and results in maintenance of MAP following resuscitation without compromising tissue perfusion in uncontrolled hemorrhagic shock.

Reportable Outcomes – Part 1

This work was the winning paper at the Northwest region of the American College of Surgeons Committee on Trauma basic science resident trauma paper competition. The paper was also presented at the national competition.

Part 2 – *Comparison of the effects of Isoflurane anesthesia and TIVA anesthesia on systemic inflammation and local mRNA production in the lung.*

This was a randomized control trial using twenty-six female Yorkshire crossbred with six swine randomized to a control arm undergoing sacrifice and tissue harvesting after induction of anesthesia. Cytokine mRNA levels from these animals served as baseline data for the population. Following induction, an 18 gauge aural intravenous (IV) catheter was placed. Animals then were switched to the blindly randomized (using a random numbers table) maintenance anesthesia consisting of either 1-3% ISO, or a TIVA regimen consisting of IV ketamine (15-33mg/kg/hr), midazolam (1-2mg/kg/hr), and buprenorphine (0.5-1 mcg/kg/hr). These doses fall within the

normal, therapeutic range for swine. The ISO group received an equivalent volume of lactated Ringer's solution (LR) instead of the IV medications to standardize the volume of fluid administered. The level of sedation was constantly monitored by an animal technician independent of the study team through measurement of jaw laxity, hemodynamic fluxuations, and response to painful stimuli at the nasal septum and forefoot. All efforts were made to ensure the study team remained blinded to the anesthetic regimen.

A left ventral cervical cut down was performed and 8F polyethylene catheters were inserted into the common carotid artery, external jugular vein, and internal jugular vein. The arterial catheter was used for continuous blood pressure analysis and blood sampling. Mean arterial pressure (MAP), and heart rate (HR) were continuously recorded and averaged every 10 seconds using a digital data collection system with a blood pressure analyzer (DigiMed, Louisville, Kentucky). The external jugular catheter was used for fluid resuscitation. The infusion of either the TIVA medications or LR (in the ISO group) was switched from the aural catheter to the internal jugular vein catheter upon its placement.

The animals underwent a midline celiotomy, suprapubic Foley catheter placement, and splenectomy. Splenectomies are performed in swine hemorrhage studies because the swine spleen in distensible and contains highly variable amounts of blood that can act as an auto-transfusion. The spleen was weighed and LR was infused to replace three times the spleen weight in grams. The abdomen was then closed with towel clamps.

Following a 15-minute stabilization period, the blood pressure was recorded and used as the baseline MAP. The abdomen was opened and residual peritoneal fluid was removed. Pre-weighed laparotomy pads were placed in both paracolic gutters and the pelvis to facilitate blood collection. A standardized Grade V liver injury (injury to a central hepatic vein) was created using a specially designed clamp. The clamp was positioned in the middle of the liver, placing the right hepatic vein, left hepatic vein, and portal vein at risk for injury. This protocol is based upon our previous studies of uncontrolled hemorrhagic shock using this same model. The time of injury was considered the start of the study (time point 0). During hemorrhage, the anesthetic regimen was stopped when the MAP was below 30 mmHg, and restarted upon rising above 30mmHg for both groups. Following 30 minutes of uncontrolled hemorrhage, the initial blood loss was determined using wall suction and the pre-weighed laparotomy pads. The abdomen was then closed. A fixed volume of LR was administered at 8ml per ml of measured blood loss at 165ml/min. This volume of fluid was calculated based upon our previous studies on the amount of LR required to maintain the baseline blood pressure for 2 hours following liver injury. The rate of administration is approximately one half the rate delivered by the Level 1 rapid infuser® as the animals were approximately one half the weight of an average human.

Upon completion of the 2-hour study period, the abdomen was reopened and the secondary blood loss was determined by adding the volume of intra-abdominal blood and the weight of the intra-abdominal blood clots. Following completion of the study the animals were sacrificed and lung tissues harvested. To ensure comparable injuries between study groups, we removed the liver post-mortem and analyzed the number of hepatic vessels injured.
Blood specimens were collected at baseline and every 30 minutes until completion of the 2-hour study. Blood assays included lactate, arterial blood gas, chemistry panel, liver function tests, and

hematocrit. Serum for cytokine analysis was collected at baseline and at study completion. Lung tissues harvested were immediately placed in RNA*later*™ solution (Ambion, Autsin, Texas) and stored at -80°C.

Results

Ten animals were randomized to each study group, and six animals were used as controls. Two animals in each study group died prior to completion of the 2 hour study period. Both study groups were similar with respect to weight, temperature, blood loss, fluid resuscitation, total urine output (UOP), and liver injury pattern. Liver function tests were within normal limits at baseline, and were similarly elevated in both groups at the end of the study ($p > 0.1$ between groups at baseline and 120 min.).

Randomized maintenance anesthesia was administered at a minimum of 60 minutes prior to liver injury, allowing equilibration of the anesthetic for each animal. Baseline MAP was 89.5mmHg for the TIVA group compared to 76.0mmHg for the ISO group ($p = 0.022$). Similarly, MAP at injury was greater in the TIVA animals than ISO animals, 86.4mmHg vs. 66.6mmHg ($p = 0.004$). Following injury there was a rapid drop in blood pressure, followed by a period of auto-resuscitation that was similar in both groups. At 30 minutes, each group received standardized fluid resuscitation resulting in a similar rise in MAP. Three animals in the TIVA group returned to their baseline blood pressure, compared with 6 in the ISO group ($p = 0.37$). Following fluid resuscitation, the MAP was maintained in TIVA animals, but persistently decreased in ISO animals. This difference became significant near the end of the study ($p < 0.05$).

Serum cytokine levels for each anesthetic group are displayed in Table 1. Within each group there is a significant elevation of IL-6, IL-8, and TNF-α at the conclusion of the study when compared to baseline. When comparing one group to another there were no differences seen at either time point ($p > 0.1$). In comparison, Figure 1 shows mRNA production in lung tissue for TIVA, ISO, and control animals as quantified with RT-PCR. Values are represented as fold change relative to the control animals, which by definition have a value of 1. While ISO animals appear to have greater IL-6 mRNA production, there is no difference when compared to TIVA animals due to the large standard deviation. Both groups do have greater expression when compared to controls ($p < 0.001$ for TIVA and ISO vs. control). For IL-8, there are no differences between all three groups. ISO animals have elevated TNF- α mRNA production when compared to both control and TIVA animals ($p = 0.004$, and $p = 0.043$, respectively). TIVA animals had similar TNF- α mRNA levels as controls ($p = 0.21$).

Table 1. Comparison of serum cytokines between TIVA and ISO groups.

		TIVA	ISO	p-value
IL-6 (pg/ml)	**Baseline**	2.2 ± 4.5	1.0 ± 1.5	0.5
	120 min.	216.8 ± 104.6	231.0 ± 162.3	0.8
	p-value	<0.001	0.005	
IL-8 (pg/ml)	**Baseline**	26.5 ± 20.1	43.2 ± 32.0	0.7
	120 min.	175.9 ± 74.4	311.0 ± 227.9	0.1
	p-value	<0.001	0.013	
TNF-α (pg/ml)	**Baseline**	52.9 ± 13.3	56.4 ± 17.1	0.2
	120 min.	118.4 ± 66.8	304.7 ± 298.5	0.1
	p-value	0.027	0.05	

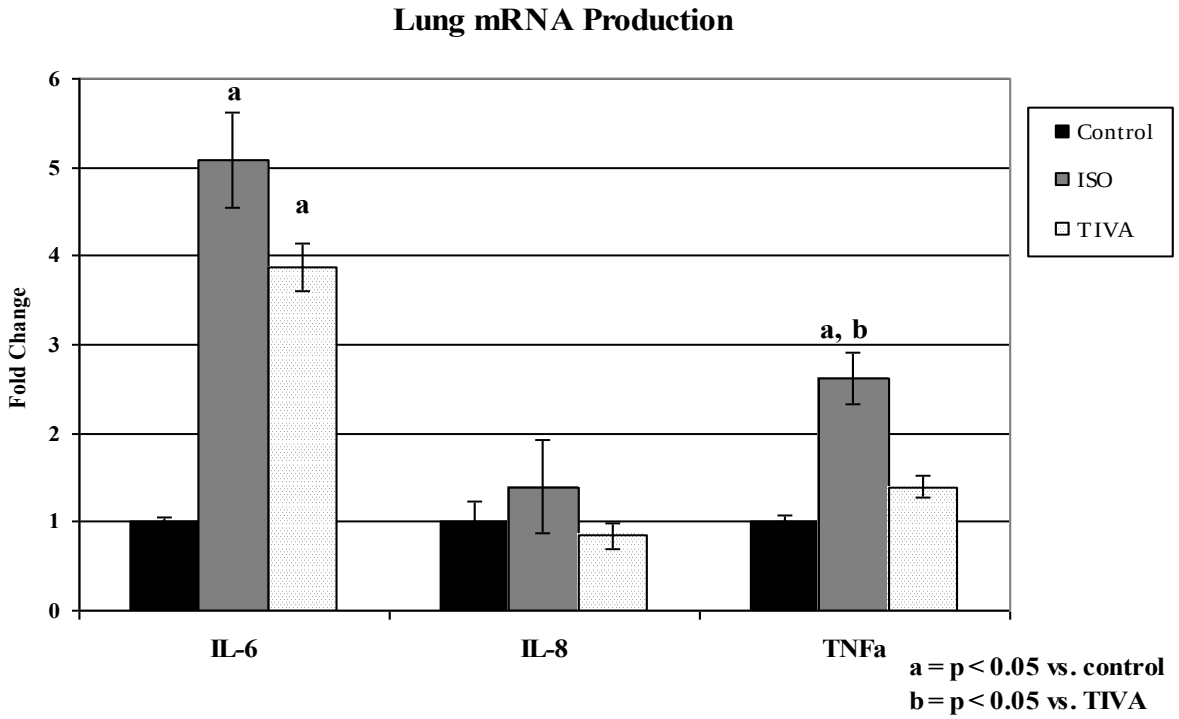


Figure 1. Lung Tissue Inflammatory Markers

Key Research Accomplishments – Part 2

1. Isoflurane and TIVA anesthesia produce similar degrees of systemic inflammation at 2 hours.
2. Anesthesia with TIVA results in suppression of TNF-alpha mRNA production in the lung compared to anesthesia with TIVA.

Reportable Outcomes – Part 2

This work was presented at the 2007 meeting of the Eastern Association for the Surgery of Trauma. The presentation was the winner of the resident competition and the manuscript won the award for the best manuscript. The work was also presented at the 2006 Portland Surgical Society and the Oregon and Washington Chapter meetings of the American College of Surgeons. It was the of the best basic science paper at the Portland Surgical Society. The abstract has been published in the Journal of Trauma and the manuscript is submitted for publication in the Journal of Trauma.

Part 3 – *Comparison of the effects of Isoflurane anesthesia and TIVA-Ketamine anesthesia on systemic inflammation and local mRNA production in the lung.*

This was a randomized controlled trial using forty female Yorkshire crossbred swine. The animals were fasted for 16 hours prior to surgery, except for water ad libitum. We pre-anesthetized the swine with an intramuscular injection of 8mg/kg Telazol® (Fort Dodge Animal Health, Fort Dodge, Iowa). All animals received midazolam (1-2 mg/kg) and buprenorphine (2-10 mg/kg) as needed to maintain adequate anesthesia. Animals randomized to receive ISO also received isoflurane for induction and animals that randomized to TIVA received a ketamine infusion during induction. Orotracheal intubation was performed with a 7.0mm or 7.5mm internal diameter cuffed endotracheal tube, and the animals were placed on mechanical ventilation. Respiratory rate and tidal volume were adjusted to keep pCO2 values between 40-50 torr. An esophageal thermometer was placed, and the animal temperature was maintained at 38.0 ± 1.5°C using external warming devices. A bispectral index monitor (BIS) was placed on the animals' head to monitor the level of anesthesia.

Eight swine (4 ISO and 4 TIVA) were randomized to a control arm and underwent sacrifice and tissue harvesting after induction of anesthesia. Cytokine mRNA levels from these animals served as baseline data for the population. Twelve animals (6 ISO and 6 TIVA) randomized to a sham group that underwent celiotomy, splenectomy and 4 hours of anesthesia.

Following induction, an 18 gauge aural intravenous (IV) catheter was placed. Animals were then switched to the blindly randomized (using a random numbers table) maintenance anesthesia consisting of either 1-3% ISO, or TIVA consisting of IV ketamine (15-33mg/kg/hr). These doses fall within the normal, therapeutic range for swine. The ISO group received an equivalent volume of lactated Ringer's solution (LR) instead of the IV medications to standardize the volume of fluid administered. The level of sedation was constantly monitored by an animal technician independent of the study team through measurement of jaw laxity, hemodynamic

fluxuations, and response to painful stimuli at the nasal septum and forefoot. All efforts were made to ensure the study team remained blinded to the anesthetic regimen.

A left ventral cervical cut down was performed and 8F polyethylene catheters were inserted into the common carotid artery, external jugular vein, and internal jugular vein. The arterial catheter was used for continuous blood pressure analysis and blood sampling. Mean arterial pressure (MAP), and heart rate (HR) were continuously recorded and averaged every 10 seconds using a digital data collection system with a blood pressure analyzer (DigiMed, Louisville, Kentucky). The external jugular catheter was used for fluid resuscitation. The infusion of either the TIVA medications or LR (in the ISO group) was switched from the aural catheter to the internal jugular vein catheter upon its placement.

The animals underwent a midline celiotomy, suprapubic Foley catheter placement, and splenectomy. Splenectomies are performed in swine hemorrhage studies because the swine spleen in distensible and contains highly variable amounts of blood that can act as an auto-transfusion. The spleen was weighed and LR was infused to replace three times the spleen weight in grams. The abdomen was then closed with towel clamps.

Following a 15-minute stabilization period, the blood pressure was recorded and used as the baseline MAP. The abdomen was opened and residual peritoneal fluid was removed. Pre-weighed laparotomy pads were placed in both paracolic gutters and the pelvis to facilitate blood collection. A standardized Grade V liver injury (injury to a central hepatic vein) was created using a specially designed clamp. The clamp was positioned in the middle of the liver, placing the right hepatic vein, left hepatic vein, and portal vein at risk for injury. This protocol is based upon our previous studies of uncontrolled hemorrhagic shock using this same model. The time of injury was considered the start of the study (time point 0). During hemorrhage, the anesthetic regimen was stopped when the MAP was below 30 mmHg, and restarted upon rising above 30mmHg for both groups. Following 30 minutes of uncontrolled hemorrhage, the initial blood loss was determined using wall suction and the pre-weighed laparotomy pads. The abdomen was then closed. LR was given at 165ml/min to achieve and maintain a MAP of 65 mmHG. The rate of administration is approximately one half the rate delivered by the Level 1 rapid infuser® as the animals were approximately one half the weight of an average human.

Upon completion of the 4-hour study period, the abdomen was reopened and the secondary blood loss was determined by adding the volume of intra-abdominal blood and the weight of the intra-abdominal blood clots. Following completion of the study the animals were sacrificed and lung tissues harvested. To ensure comparable injuries between study groups, we removed the liver post-mortem and analyzed the number of hepatic vessels injured.

Blood specimens were collected at baseline and every 60 minutes until completion of the 4-hour study. Blood assays included lactate, arterial blood gas, chemistry panel, liver function tests, and hematocrit. Serum for cytokine analysis was collected at baseline and at study completion. Lung tissues harvested were immediately placed in RNA*later*™ solution (Ambion, Autsin, Texas) and stored at -80°C.

Part 3 - Results

Demographics are presented in Table 1. There was no difference in estimated blood loss (EBL), resuscitation volume or urine output between groups. More animals in the ketamine/injury arm died prematurely (5 of 10) than in the isoflurane/injury arm (0 of 10) ($p = 0.03$). The five animals that died prematurely had a significantly higher blood loss ($p < 0.001$). There was no difference in blood loss between the surviving ketamine/injury animals and the isoflurane/injury animals.

There was no difference in the pre- or post-injury mean arterial pressures, heart rate or peripheral oxygenation between groups. Mean arterial pressures are compared between groups in Figure 1. As shown in the figure, animals that died in the ketamine group had a lower MAP at baseline and they died very quickly after injury.

There was no difference in lactate values or base deficit between groups. (Figure 2) Animals receiving ketamine had a significantly higher BIS score ($p < 0.01$) and a higher serum sodium level ($p < 0.05$) at baseline, after injury and after resuscitation.

Serum cytokines levels, measured at the end of the study, are shown in Figure 3. There was no difference in levels for IL-6. IL-8 and TNF-a levels were significantly greater in animals receiving TIVA than those receiving ISO. Lung tissue cytokine mRNA fold changes are shown in Figure 4. Fold change of controls is defined as 1. Fold change was greater for TNF-a in the TIVA group but not different for the other 2 cytokines.

Table 1. Demographics

	Ketamine (n=10)	**ISO (n=10)**	**p**
Weight	36.23 ± 4.6 kg	35.38 ± 2.8 kg	0.63
Survival	5	10	0.03
EBL after injury (ml)	987.9 ± 315.5	1047.4 ± 267.5	0.71
EBL per kg per min alive (ml)	0.16 ± 0.05 (alive) (3.02 ± 3.06 [dead])	0.18 ± 0.05	0.28 (> 0.10)
Resuscitation volume (ml)	5588 ± 3300	6990 ± 4137	0.52
Total UOP (ml)	520.0 ± 445.6	389.0 ± 285.4	0.50
Total UOP per kg (ml)	14.6 ± 13.5	10.8 ± 7.3	0.49

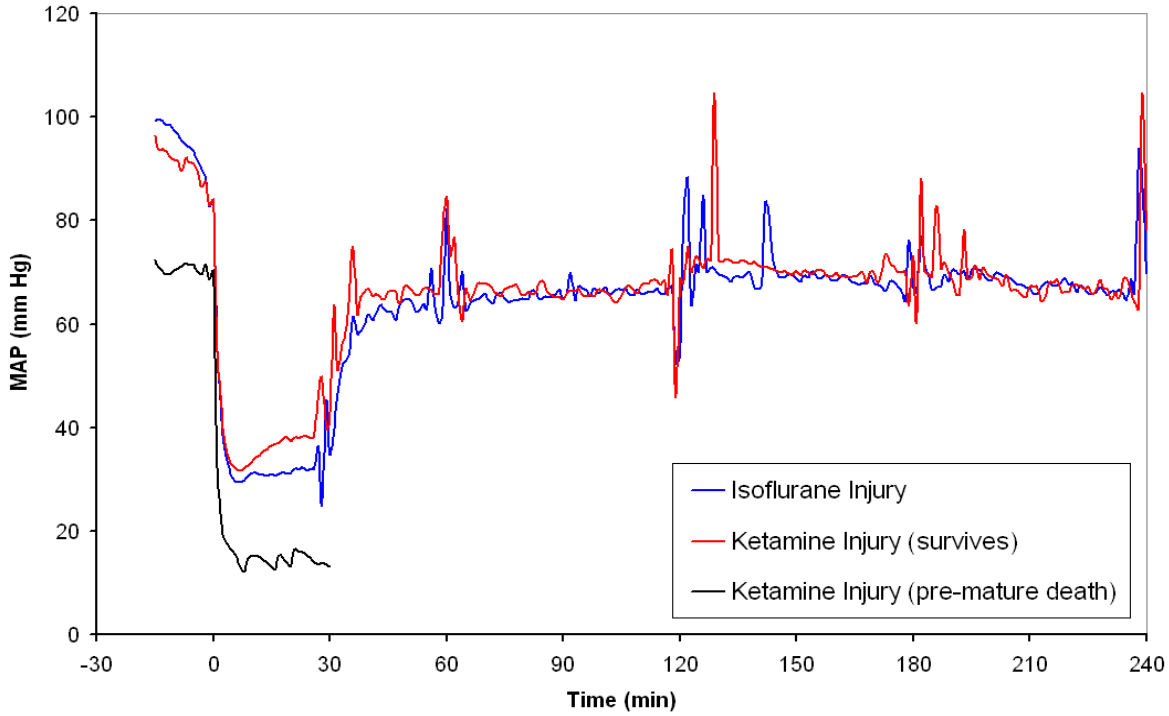


Figure 1. MAP compared between groups

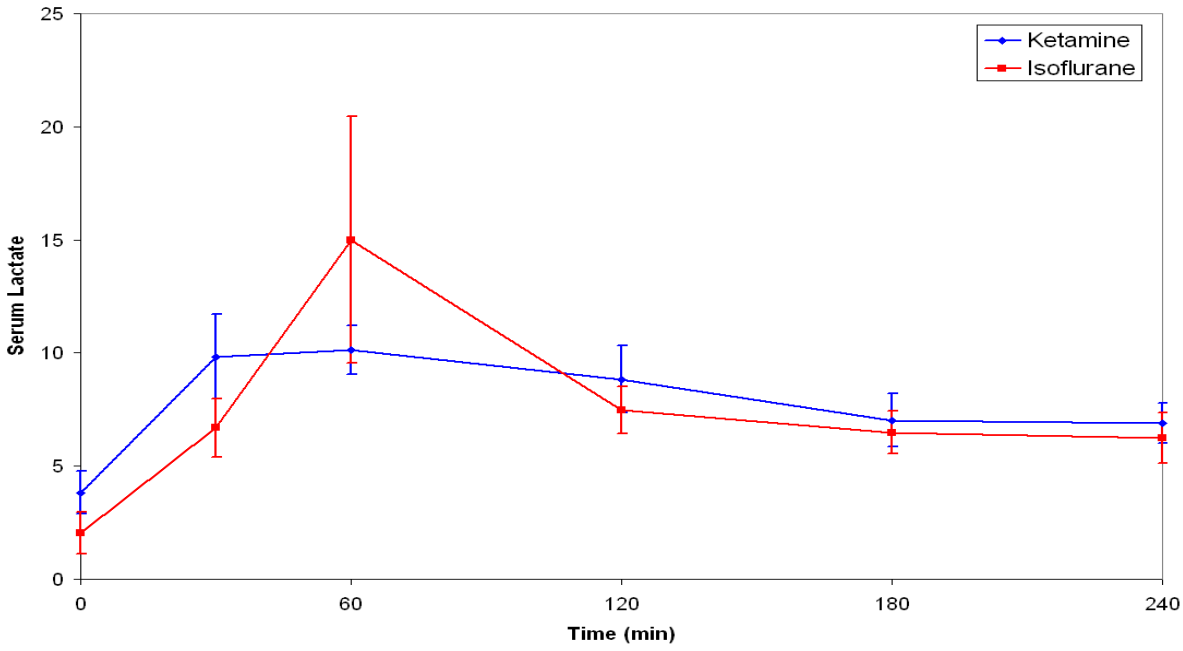


Figure 2. Lactate compared between groups

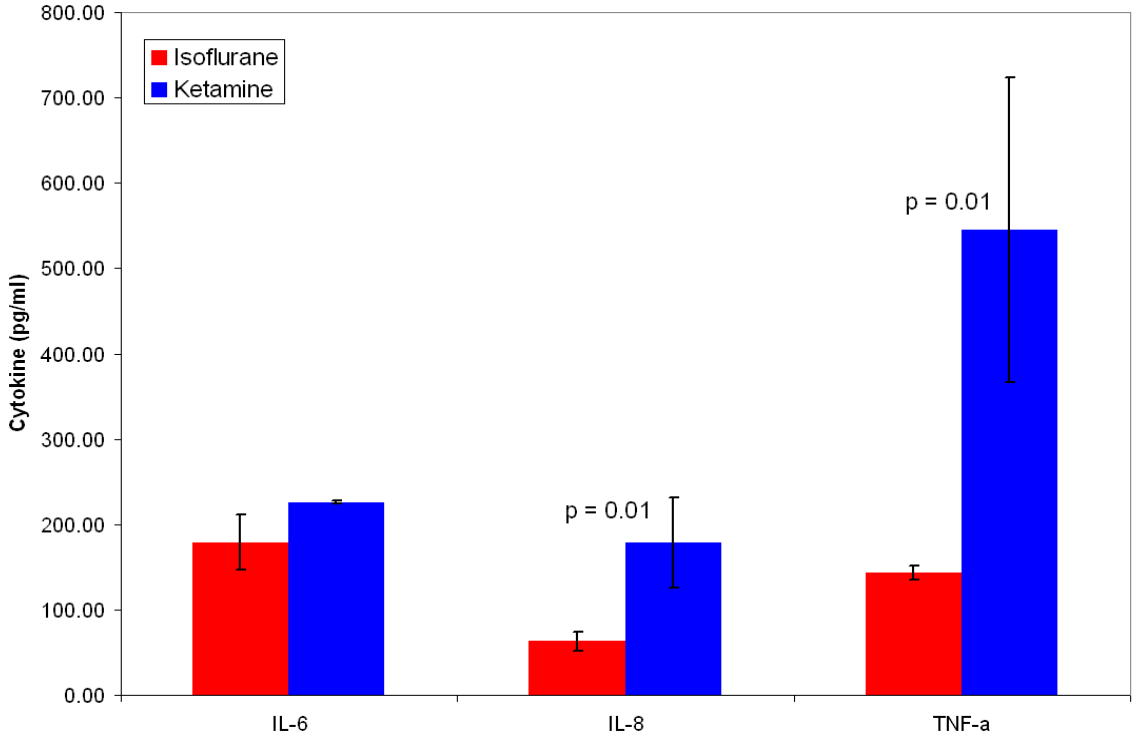


Figure 3. Serum cytokine levels

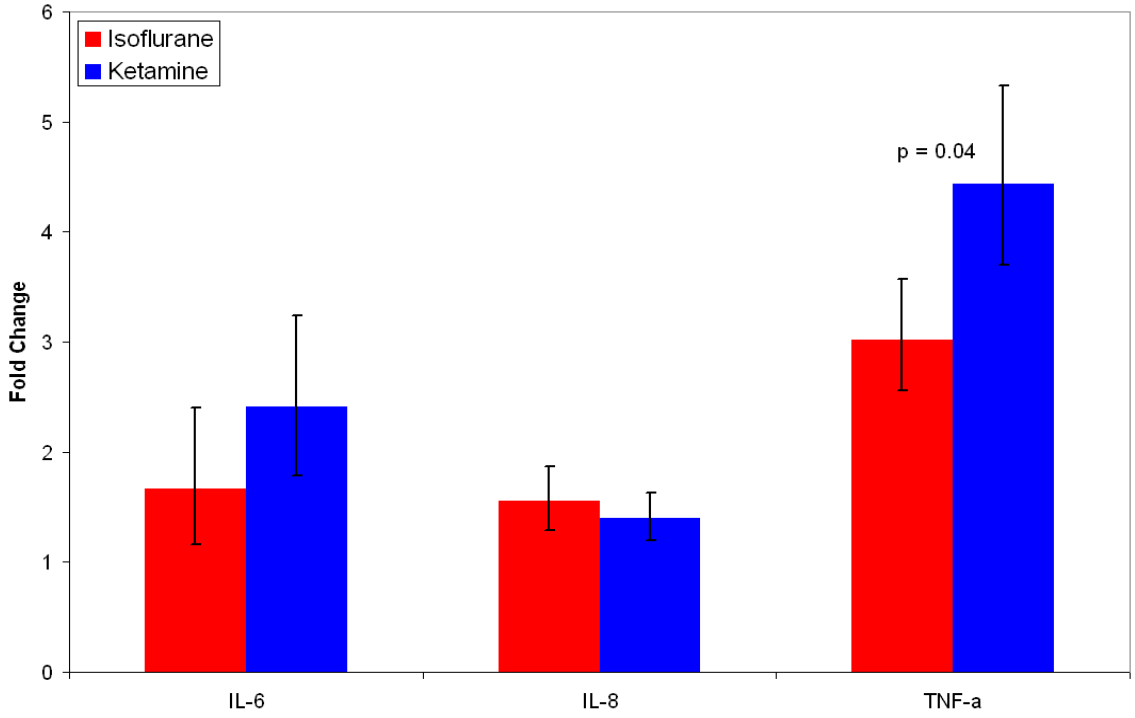


Figure 4. Lung tissue cytokine mRNA fold change

Key Research Accomplishments – Part 3

1. The exclusive use of TIVA results in increased mortality compared to isoflurane in a Grade V liver injury model in swine.
2. The exclusive use of TIVA results in increased dysfunctional inflammation measured in serum cytokines measured at 4 hours after injury.
3. The exclusive use of TIVA results in increased dysfunctional inflammation measured in lung tissue.

Reportable Outcomes – Part 3

This research was presented at the 2007 Region X Residents' Competition of the American College of Surgeons Committee on Trauma. The work was also presented at the 2007 combined meeting of the Association of Academic Surgeons and the Society of University Surgeons.

Specific Aim 8 – Comparison of currently military resuscitation fluids

Materials and Methods

This study was designed as a prospective, randomized, blinded trial in a swine model that took place in a Level 1 Trauma Center animal laboratory. The model was developed at the Oregon Health & Science University (OHSU), and approved by the Institutional Animal Care and Use Committee. This facility adheres to the National Institutes of Health guidelines for the care and use of laboratory animals.

Female Yorkshire Crossbred swine underwent the following protocol to determine the efficacy of an initial bolus of resuscitative fluids currently utilized in military and civilian settings on physiologic response to uncontrolled hemorrhagic shock. It was hypothesized that resuscitation with normal saline would result in inferior outcomes compared to other commonly used resuscitation fluids.

Fifty similarly sized Yorkshire-crossbred female swine underwent a 16 hour pre-operative fast except for water ad libatum. The swine were pre-anesthetized with an intramuscular injection of 8 mg/kg Telazol (Fort Dodge Animal Health, Fort Dodge, IA) and then intubated orally with a 7.0 to 7.5 mm endotracheal tube. Animals were placed on mechanical ventilation and constantly monitored by an independent animal technician who adjusted the respiratory rate to maintain the pCO_2 between 40 to 50 mm Hg. Anesthesia was maintained with 2% isoflurane in 100% oxygen with the animal technician monitoring jaw laxity to assess anesthesia adequacy. An esophageal monitor was placed and euthermia was obtained utilizing external warming devices.

After swine were anesthetized, additional monitoring devices were placed. An InSpectra Tissue Spectrometer (Hutchinson Technology, Hutchinson, MN) was placed on the left hind limb of the swine for continuous monitoring of tissue oxygen saturation (StO_2) throughout the experiment. Left cervical cutdowns were performed, and polyethylene catheters were inserted respectively into the left common carotid and left external jugular vein. The arterial line was utilized for

continuous blood pressure monitoring, and mean arterial pressure (MAP) was continuously recorded and averaged every 10 seconds with a blood pressure analyzer and digital data collection system (DigiMed, Louisville, KY). The venous line was used for administration of bolus resuscitation fluids. Finally, a left femoral cutdown was performed, and the left femoral artery was cannulated for blood sampling throughout the experiment.

After placement of these monitoring devices, a midline celiotomy and suprapubic Foley catheterization were performed. Following a 15-minute stabilization period, residual peritoneal fluid was removed from the abdominal cavity. Pre-weighed laparotomy sponges were placed in the left and right pericolic gutters as well as the pelvis to facilitate primary hemorrhage collection. Then, a standardized Grade V liver injury (injury to a central hepatic vein, consistent with the AAST scaling and scoring system) was created using a specially designed clamp with 4 razor sharp edges. This protocol for liver injury has been previously validated in our prior studies of uncontrolled hemorrhagic shock. The time of injury was considered the start time for the 2 hour experimental time period.

Animals were allowed to hemorrhage for 30 minutes during which time the primary blood loss was collected with wall suction and by the three previously placed pre-weighed laparotomy sponges. After 30 minutes, the three laparotomy sponges were removed, and the liver was then packed with six pre-weighed laparotomy sponges in a manner consistent with a damage control operation in order to collect secondary blood loss. The abdomen was then temporarily closed with penetrating towel clamps.

Animals were randomized to 4 different groups for bolus fluid resuscitation, which was initiated 30 minutes after injury. The fluids administered included fluids and associated volumes currently utilized for bolus resuscitation in both civilian and military practice as follows: 2 liters of NS, 2 liters of LR, 500 ml of Hextend (HEX), and 250 ml of 7.5% hypertonic saline with 3% Dextran (HTS). A no fluid (NF) arm acted as a control group. Ten animals were randomized to each respective fluid/control group. Surgical staff was blinded to fluid resuscitation, as all fluids were administered over a 12-minute time period from a sterile opaque container which was previously filled by an independent technician who controlled fluid rate ml/min to achieve resuscitation in the 12 minute allotted time period regardless of fluid type. Surgical staff was only aware of the control/no fluid group randomization after creation of the standardized injury and temporary closure of the abdominal cavity.

The animals that did not die from exsanguination during the course of the experiment were sacrificed at the completion of the 2-hour study with a euthanasia solution. Then, the six pre-weighed laparotomy sponges placed prior to abdominal closure were removed and weighed to determine secondary blood loss. Lung tissue was collected at the end of 2 hours or at declaration of death for rtPCR analysis. Tissue was stored in RNA later and a 10% buffered formalin solution. An autopsy of the liver was performed to ensure comparable injuries without portal vein involvement.

Study Variables

Physiologic variables included survival, MAP, StO_2, blood loss from the uncontrolled hemorrhage, and blood loss post liver injury. Laboratory values include Hct, lactate, ABG,

chemistry panel, and complete blood count (CBC). Coagulation parameters include the PT, PTT, ACT, and Factors II, V, VII, VIII, IX, X, XI, XII, Protein C, Protein S. Serum was collected for analysis of inflammatory cytokines at time points baseline, one-hour and end of study.

Results

Physiologic and Laboratory Analysis

Ten animals were randomized to each group. The baseline weight of swine in all groups was similar (Table 1). Two animals in the NF group did not survive to study completion; however, their data is included in the analysis up until the time of their deaths. There was no statistically significant difference in survival between groups, $p = 0.50$. All animals had similar Grade V liver injuries without portal vein injury as determined by autopsy. Primary blood loss before resuscitation was similar for all groups (Table 1). Secondary blood loss after resuscitation is presented in Table 1. The secondary blood loss in the NF group was significantly lower than all other groups, $p < 0.01$. Though the NS and LR groups had similar secondary blood losses, which were higher than both the HEX and HTS groups, none of these groups were statistically different from one another.

Table 1. Baseline demographics and secondary blood loss data. Weight and primary blood loss presented as mean ± SEM. Secondary blood loss presented as median with IQR. [a] indicates significantly less than NS, LR, HEX, and HTS, $p < 0.01$.

Fluid	Weight	Primary blood loss	Survival	Secondary blood loss
NS	35.1 ± 0.6	1063.9 ± 103.9	10	138.5 (83.1, 169.6)
LR	34.5 ± 0.4	794.6 ± 82.3	10	127.5 (118.5, 134.5)
HEX	35.0 ± 0.6	870.5 ± 103.3	10	92.0 (77.4, 190.5)
HTS	35.9 ± 1.0	981.4 ± 126.5	10	99.1 (82.6, 145)
NF	34.2 ± 0.4	956.2 ± 139.9	8	62.3 (37.3, 80.1)[a]

Laboratory values for hematocrit (Hct) are presented in Table 2. At both the 1 and 2-hour time points, the NF group had significantly higher Hcts compared to the other groups, $p < 0.01$. The LR group had a significantly higher Hct compared to the NS, HEX, and HTS groups at 1 hour and compared to the HEX and HTS groups at 2 hours, all $p < 0.03$. Additionally, the NS group had a significantly higher Hct compared to the HEX group at 2 hours, $p < 0.02$.

Table 2. Hematocrit (Hct) data. Values presented as mean ± SEM. [a] indicates significantly > NS, LR, HEX, and HTS. [b] indicates significantly > NS, HEX, and HTS. [c] indicates significantly > HEX and HTS. [d] indicates significantly > HEX. For all significant comparisons, $p < 0.03$.

		Hct	
Fluid	Base	1 HR	2 HR
NS	30.1 ± 0.7	20.0 ± 1.0	23.8 ± 0.8[d]
LR	29.8 ± 0.6	23.4 ± 0.8[b]	26.7 ± 1.2[c]
HEX	28.9 ± 0.7	20.6 ± 0.7	21.1 ± 0.7
HTS	30.0 ± 0.7	20.9 ± 0.5	23.3 ± 0.8
NF	29.5 ± 0.8	32.0 ± 0.9[a]	32.6 ± 1.0[a]

Continuous MAP data for all groups is presented in Figure 1. All animals experienced a significant initial drop in MAP during the uncontrolled hemorrhage period, followed by a period of spontaneous blood pressure elevation. At 30 minutes, bolus fluid resuscitation resulted in a significant increase in MAP, after which, the MAP decreased over the rest of the study period. The observed increase in MAP in the NF group at 70 and 75 minutes was secondary to the death of animals in this group at these two time points.

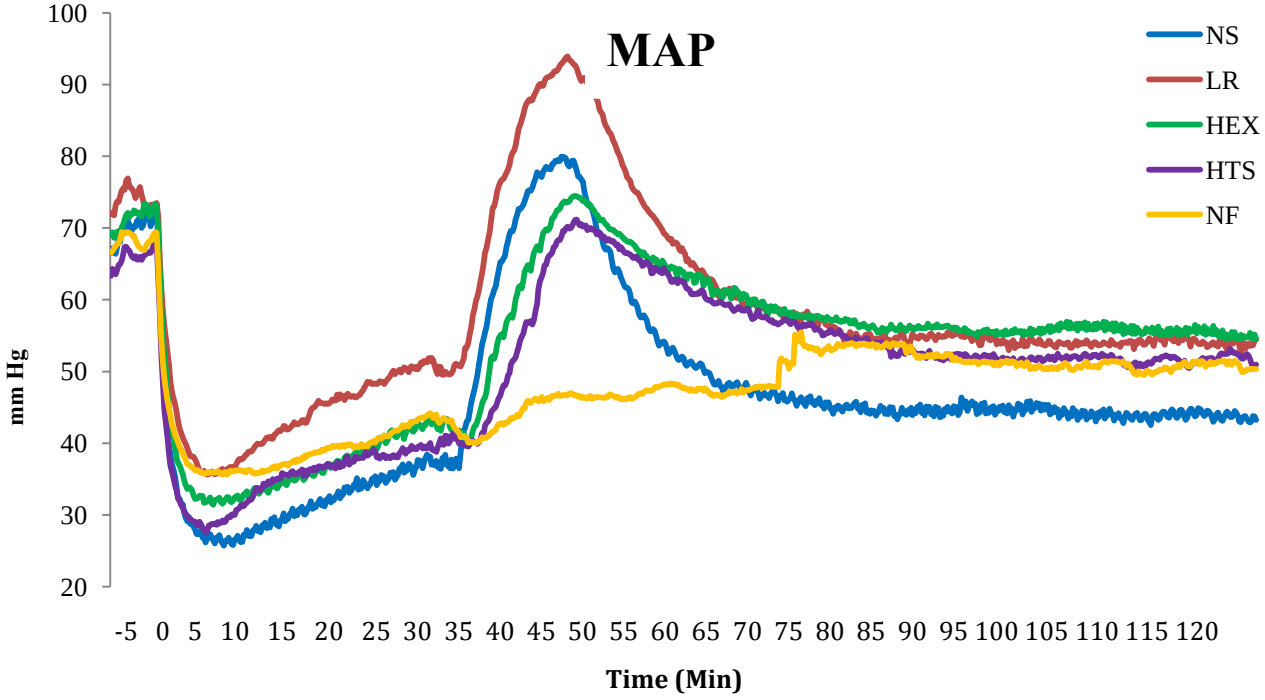


Figure 1. Continuous Mean Arterial Pressure (MAP). Data presented are the average MAP for each group at each time point. Liver injury was created at time point 0. Fluid admin began at time point 30 min.

MAP data at baseline, 1 hour, and 2 hours are presented in Table 3. At 1 HR, both the NS group and the NF group had a significantly lower MAP compared to the LR, HEX, and HTS groups, all $p < 0.05$. At 2 HR, the MAP in the NS group was significantly lower than the LR, HEX, and HTS groups, all $p < 0.04$. The MAP did not differ between the LR, HEX, and HTS groups at 1 or 2 HRS. The MAP of the NF group was not different from the other groups at 2 HRS.

		MAP			**StO_2**	
Fluid	Base	1 HR	2 HR	Base	1 HR	2 HR
NS	68.5 ± 4.4	50.1± 2.9[a]	43.3 ± 2.7[a]	76.3 ± 2.7	76.8 ± 3.7	69.7 ± 2.5 [c]
LR	73.2 ± 4.5	63.3 ± 4.4	54.5 ± 3.4	75.8 ± 2.1	83.7 ± 2.1	74.5 ± 2.1
HEX	70.7 ± 4.1	62.8 ± 2.1	54.6 ± 2.5	72.4 ± 1.6	78.5 ± 3.2	77.8 ± 3.4
HTS	69.0 ± 2.9	60.0 ± 3.0	51.1 ± 2.2	73.6 ± 1.7	79.9 ± 1.0	77.1 ± 1.6
NF	71.4 ± 3.3	47.5 ± 4.8[a]	46.6 ± 5.3	69.7 ± 3.6	59.6 ± 5.6[b]	67.3 ± 3.2[a]

Table 3. MAP and StO_2 data at baseline, 1 hour, and 2 hours. Values presented as mean ± SEM. [a] indicates significantly < LR, HEX, and HTS. [b] indicates significantly < NS, LR, HEX, and HTS. [c] indicates significantly < HTS. For all significant comparisons, $p < 0.05$.

Continuous StO_2 data for all groups is presented in Figure 2, with a similar curve as previously noted with MAP. StO_2 data at baseline, 1 hour, and 2 hours is presented in Table 3. At 1 hour, the NF group had a significantly lower StO_2 compared to the other groups, $p < 0.04$. At 2 hours, the StO_2 in the NF group was lower than the LR, HEX, and HTS groups, all $p < 0.04$. Also at 2 hours, the StO_2 in the NS group was lower than the HTS group, $p = 0.02$. There was no difference between the NF and NS groups at 2 hours. StO_2 did not differ between the LR, HEX, and HTS groups at 1 hour or 2 hours.

Laboratory values for pH are presented in Table 4. Swine are alkalotic at baseline, which has been observed in prior experiments. At 1 hour, there was no difference between groups. At 2 hours, the NS group had a significantly lower pH compared to the LR, HEX, and NF groups, all $p < 0.02$. Also at 2 hours, the HTS group had a significantly lower pH compared to the LR and HEX groups, all $p < 0.05$. Lactate values are also shown in Table 4. All groups demonstrated a significant increase in lactate from baseline to 1 hour, all $p < 0.01$. At 2 hours, there was a significant decrease in lactate levels from the 1 hour time point in the LR, HEX, HTS, and NF groups, all $p < 0.04$. However, there was no significant decrease from 1 hour to 2 hours in the NS group, $p = 0.14$. There were no significant differences between fluid groups at any time point.

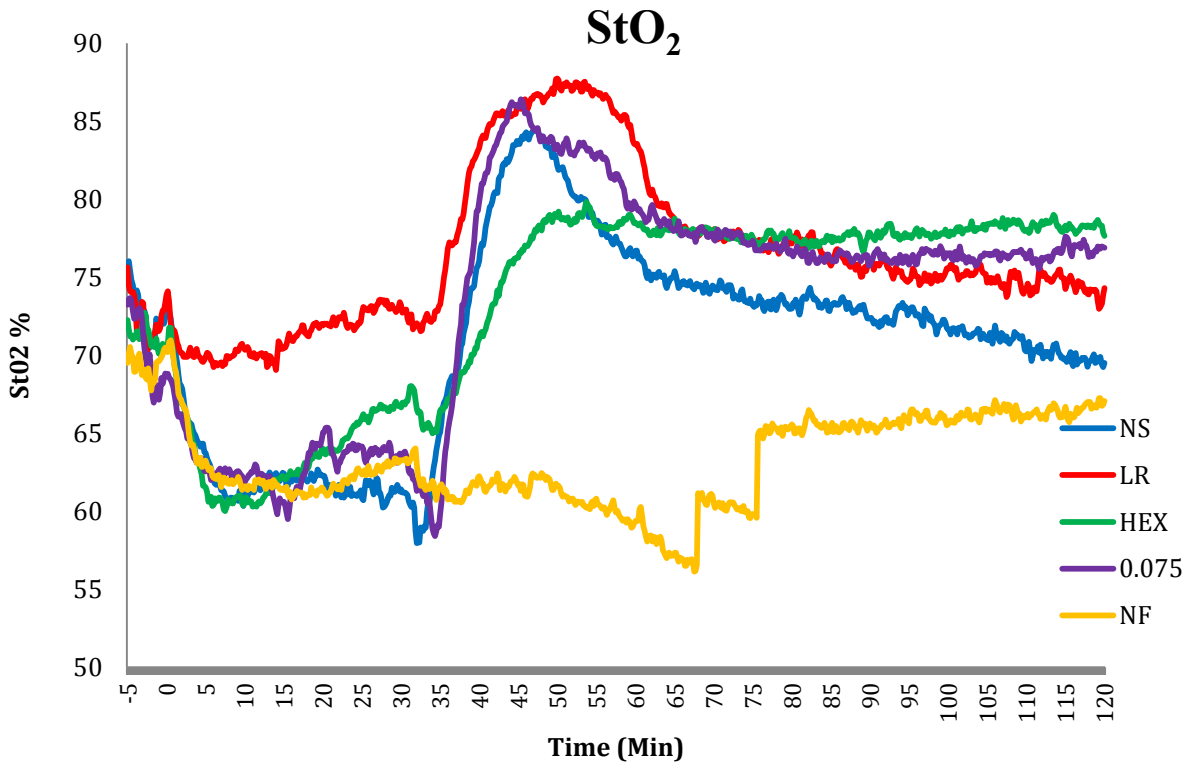


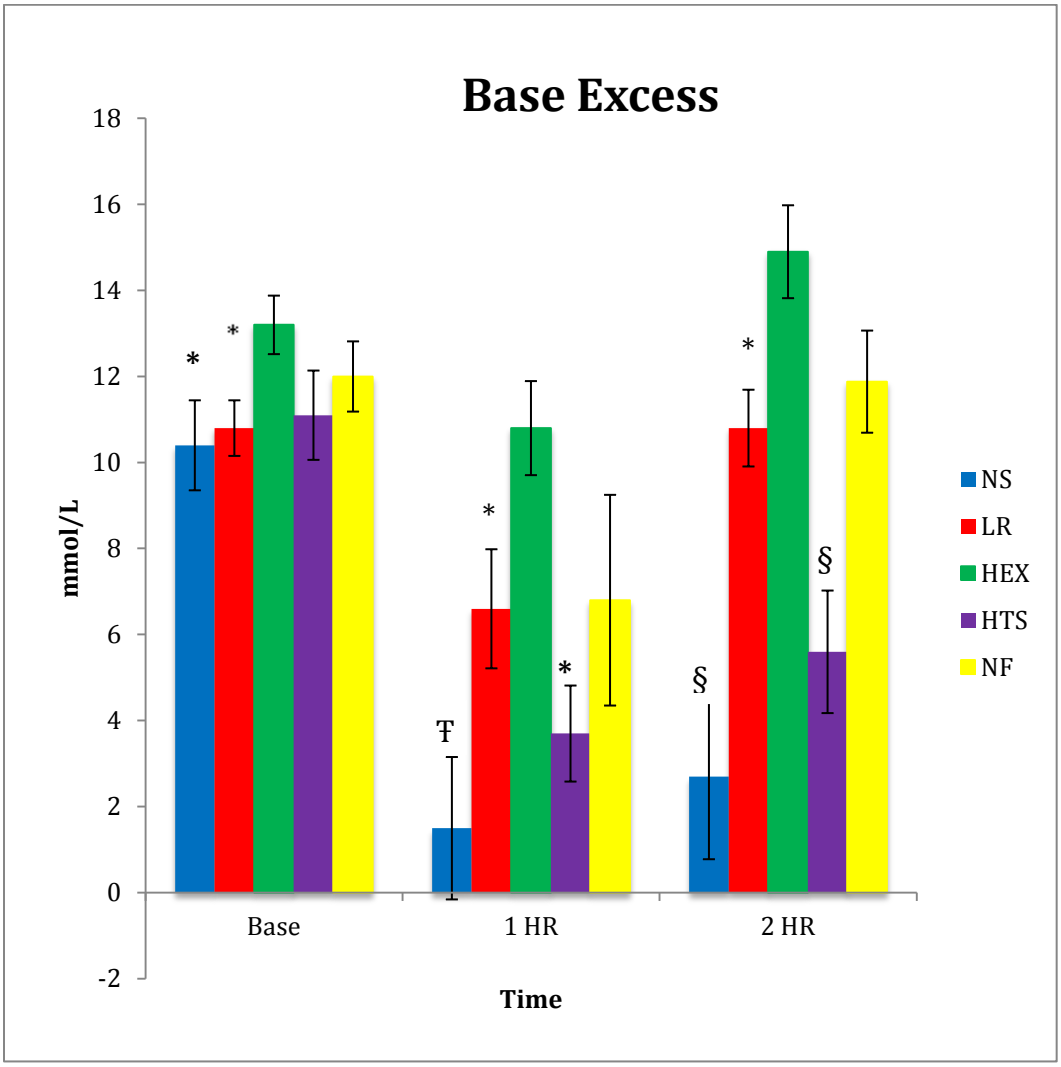
Figure 2. Continuous tissue oxygen saturation (StO_2). Data presented are the average StO_2 for each group at each specific time point. Liver injury was created at time point 0. Fluid admin began at time point 30 min.

		pH			Lactate	
Fluid	Base	1 HR	2 HR	Base	1 HR	2 HR
NS	7.54 (7.48, 7.59)	7.43 (7.36, 7.49)	7.43 (7.35, 7.51) [a]	1.9 (1.4, 2.3)	3.2 (2.5, 6.6)*	2.3 (1.8, 5.9)
LR	7.54 (7.51, 7.58)	7.47 (7.42, 7.53)	7.54 (7.49, 7.57)	1.8 (1.6, 2.4)	4.7 (3.3, 6.3)*	3.3 (2.1, 3.8)[Ŧ]
HEX	7.56 (7.53, 7.58)	7.51 (7.42, 7.56)	7.57 (7.50, 7.60)	1.4 (1.1, 2.2)	3.6 (2.7, 4.9)*	2.3 (2.0, 2.9)[Ŧ]
HTS	7.53 (7.46, 7.59)	7.44 (7.36, 7.48)	7.48 (7.37, 7.53) [b]	2.0 (1.5, 2.6)	3.6 (2.7, 4.9)*	2.8 (2.0, 3.9)[Ŧ]
NF	7.56 (7.50, 7.58)	7.49 (7.42, 7.53)	7.52 (7.50, 7.54)	2.2 (1.7, 2.5)	3.4 (2.7, 7.1)*	2.6 (2.4, 3.5)[Ŧ]

Table 4. pH and lactate labs presented as median with IQR. [a] indicates significantly < LR, HEX, and NF. [b] indicates significantly < LR and HEX. * indicates a significant increase in lactate from baseline to 1 hour in each respective group. [Ŧ] indicates a significant decrease in lactate from 1 hour to 2 hours in each respective group. There were no significant differences in lactate between groups. ($p < 0.05$.)

Laboratory values for base excess are shown in Figure 3. The NS group had a significantly lower base excess compared to the LR group at 1 hour and 2 hours, the HEX group at all time points, and the NF group at 2 hours, all $p < 0.03$. The HTS group had lower base excess than the HEX group at 1 hour and the LR, HEX, and NF groups at 2 hours, all $p < 0.01$. The LR group had a significantly lower base excess compared to HEX at all time points, all $p < 0.03$.

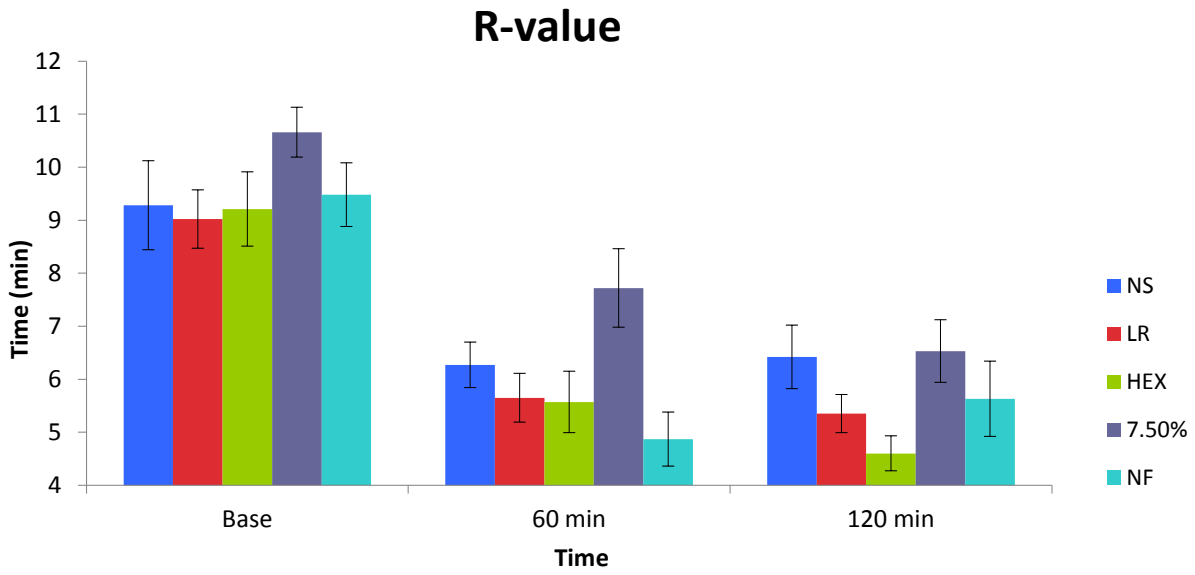
Figure 3. Base excess data. Values presented as mean ± SEM. * indicates significantly < HEX. Ŧ indicates significantly < HEX and LR. § indicates significantly < HEX, LR, and NF. ($p < 0.05$)



Coagulation

R-value data are presented in Figure 4. All groups demonstrated a significant decrease in R-values from baseline to 1 hour and baseline to 2 hours, $p < 0.02$. The HTS group had a significantly higher R-value compared to the LR, HEX, and NF groups at 1 hour, all $p < 0.04$. The HEX group had a significantly lower R-value compared to NS and HTS at 2 hours, $p < 0.02$.

Figure 4. R-value data. Values presented as mean ± SEM. ¶ indicates $p < 0.05$ compared to baseline value. # indicates $p < 0.05$ compared to LR, HEX, and NF. Ŧ indicates $p < 0.05$ compared to NS and HTS.



MA data are presented in Figure 5. The NF group had a significantly higher MA compared to the NS, HEX, and HTS groups at 1 hour and 2 hours, $p < 0.05$. The LR group had a significantly higher MA compared to the HEX and HTS groups at 1 hour and compared to the NS, HEX, and HTS groups at 2 hours, all $p < 0.05$. The NS group had a significantly higher MA compared to the HTS group at 2 hours, $p < 0.05$.

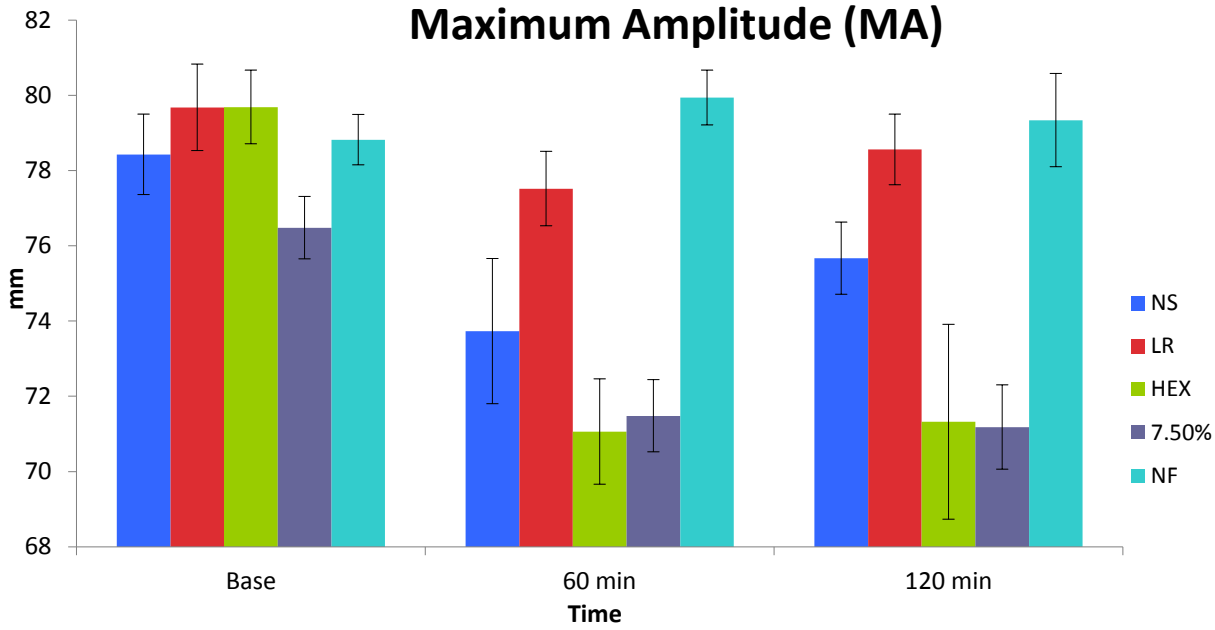


Figure 5. MA data. Values presented as mean ± SEM. Ŧ indicates significantly > NS, HEX, and HTS. # indicates significantly > HEX and HTS. ¶ indicates significantly > HTS. ($p < 0.05$)

The α angle data were also compared. The LR group had a significantly greater α angle compared to HEX and HTS at 1 hour, both $p < 0.05$. The NF group also had a significantly greater α angle compared to HEX and HTS at 1 hour, both $p < 0.05$.

Routine coagulation parameters were collected at baseline, 1 hour, and 2 hours. The difference between baseline, 1 hour and 2 hour values were calculated (baseline minus 1 hour value or baseline minus 2 hour value), and data are presented as the delta values (Δ). Thus, a negative delta value indicates an overall increase in the coagulation parameter, whereas a positive delta value indicates an overall decrease in the coagulation parameter.

The ΔPT data are presented in Table 5. The NS, LR, HEX, and HTS groups had significantly greater increases in PT from baseline compared to NF at 1 hour. In addition, the NS, HEX, and HTS groups had significantly greater increases in PT from baseline compared to NF at 2 hours. The NS and HTS groups had significantly greater increases in PT from baseline compared to the LR group at 1 hour, while NS, HEX, and HTS groups had significantly greater increases in PT from baseline compared to the LR group at 2 hours, all $p < 0.05$.

Fluid	ΔPT 1 (seconds)	ΔPT 2 (seconds)
NS	-2.1 ± 0.2^{ab}	-1.6 ± 0.6^{ab}
LR	-0.8 ± 0.2^{a}	-0.3 ± 0.2
HEX	-1.4 ± 0.3^{a}	-0.9 ± 0.2^{ab}
HTS	-1.9 ± 0.2^{ab}	-1.4 ± 0.2^{ab}
NF	0.2 ± 0.2	0.2 ± 0.2

Table 5. Delta PT data. Values presented as mean ± SEM. ΔPT 1 signifies baseline PT minus 1 hour PT value; ΔPT 2 signifies baseline PT minus 2 hour PT value. [a] indicates a significantly greater increase from baseline compared to NF at that time point. [b] indicates a significantly greater increase from baseline compared to LR at that time point ($p < 0.05$).

The Δ fibrinogen data are presented in Table 6. The NS, LR, HEX, and HTS groups had significantly greater decreases in fibrinogen from baseline compared to the NF group at 1 hour and at 2 hours, all $p < 0.05$. There were no differences in ΔPTT values at 1 or 2 hours.

Fluid	ΔFib 1	ΔFib 2
NS	67.9 ± 5.1[a]	62.3 ± 6.1[a]
LR	49.0 ± 8.5[a]	38.6 ± 9.9[a]
HEX	54.7 ± 6.2[a]	55.4 ± 5.0[a]
HTS	59.0 ± 3.8[a]	47.7 ± 8.9[a]
NF	10.5 ± 5.6	5.4 ± 7.0

Table 6. Delta fibrinogen data. Values presented as mean ± SEM. ΔFib 1 signifies baseline fibrinogen minus 1 hour fibrinogen value; ΔFib 2 signifies baseline fibrinogen minus 2 hour fibrinogen value. [a] indicates a significantly greater decrease in fibrinogen level from baseline compared to NF at that time point. ($p < 0.05$)

Key Research Accomplishments:

1. Baseline characteristics similar
2. Animals receiving NF had less secondary blood loss
3. 1 hour post injury MAP for LR, HEX and HTS is significantly better than NS or NF
4. 2 hour post injury MAP only NS was significantly lower / worse than all other fluids
5. HEX provides more consistent StO_2 compared to other fluids
6. In clinically utilized bolus volumes, HEX, HTS and LR are similar in all study with respect to all study parameters
7. 250 ml bolus of HTS provides equivalent resuscitation to the larger dosing regimens but results in a more relatively hypocoagulable state
8. NF provides a similar outcome to NS with a less acidotic state and decreased secondary blood loss. This makes one question if NS should be utilized as an initial fluid resuscitation.
9. Withholding fluid results in less change in coagulation parameters and post treatment blood loss.

Reportable Outcomes

This data was presented at the Region X Committee on Trauma Competition. This data was awarded first prize at the Oregon chapter of Society of Critical Care Medicine. A presentation was given at this meeting in Vancouver, WA. In addition, an abstract was presented at the 2011 annual meeting of Pacific Coast Surgical Association in Scottsdale, AZ. It was also selected to compete for the Resident competition award at that meeting. This data was published in the Journal of Trauma. In addition, this data was presented at the 97th Annual American College of Surgeons National Meeting in San Francisco, CA. The coagulation data from that presentation has been sent to the Journal of American College of Surgeons for review.

Bibliography of study generated work over the course of the grant

MANUSCRIPTS

1. Watters JM, Brundage SI, Todd SR, Zautke NA, Stefater JA, Lam JC, Muller PJ, Malinoski D, **Schreiber MA**. Resuscitation with Lactated Ringer's Does Not Increase Inflammatory Response in a Swine Model of Uncontrolled Hemorrhagic Shock. Shock. 2004; 22:283-287.

2. Brundage SI, Zautke NA, Watters JM, Stefater JA, Todd SR, Lam JC, **Schreiber MA.** Lactated Ringer's Does Not Increase Inflammation after Shock. 6th World Congress on Trauma, Shock, Inflammation and Sepsis. 2004; EC302C0210:151-155.

3. Watters JM, Jackson T, Muller PJ, Malinoski D, Todd SR, **Schreiber MA**. Fluid Resuscitation Increases Inflammatory Response to Traumatic Injury. Journal of Trauma. 2004; 57:1378.

4. Watters JM, Differding JA, Muller PJ, **Schreiber MA**. A Single Bolus of 3% Hypertonic Saline with Dextran Provides Optimal Resuscitation after Uncontrolled Hemorrhagic Shock. Journal of Trauma. 2005; 58:216.

5. Todd SR, Malinoski DJ, Muller PJ, **Schreiber MA**. Hextend Attenuates Hypercoagulability Following Severe Injury in Swine. Journal of Trauma. 2005:59;589-594.

6. Kiraly LN, Differding JA, Enomoto TM, Sawai RS, Muller PJ, Diggs B, Tieu BH, Englehart MS, Underwood S, Wiesberg TT, **Schreiber MA**. Resuscitation with Normal Saline (NS) vs. Lactated Ringers Modulates Hypercoagulability and Leads to Increased Blood Loss in an Uncontrolled Hemorrhagic Shock Swine Model. Journal of Trauma. 2006:61;57-65.

7. Watters JM, Tieu BH, Differding JA, Muller PJ, **Schreiber MA**. A Single Bolus of 3% Hypertonic Saline with 6% Dextran Provides Optimal Initial Resuscitation after Uncontrolled Hemorrhagic Shock. Journal of Trauma. 2006:61;75-81.

8. Watters JM, Tieu BH, Todd SR, Jackson T, Muller PJ, Malinoski DJ, **Schreiber MA.** Fluid Resuscitation Increases Inflammatory Gene Transcription Following Traumatic Injury. Journal of Trauma. 2006;61;300-309.

9. Todd SR, Malinoski DJ, Muller PJ, **Schreiber MA**. Lactated Ringer's is Superior to Normal Saline in the Resuscitation of Hemorrhagic Shock. Journal of Trauma. 2007;62:636-639.

10. Tieu BH, Enomoto TM, Phillips C, **Schreiber MA** et al. Resuscitation with Normal Saline Increases Volume Requirement, Increases Cardiac Output and Decreases Systemic Vascular Resistance Compared to Lactated Ringers in an Uncontrolled Hemorrhagic Shock Model in Swine. JACS. 2006 Sept; 203(3) supplement: S32.

11. Englehart MS, AlTieu BH, Kiraly LN, Underwood SA, Muller PJ, Sawai RS, **Schreiber MA**. Ketamine-Based Total Intravenous Anesthesia is Superior to Isoflurane in a Swine

Model of Hemorrhagic Shock. Journal of Trauma. 2008 Oct;65(4): 901-8; discussion 908-09.

12. Gee AC, Englehart MS, Tieu BH, Cho SD, Morris M, Karahan A, Underwood S, Muller PJ, and **Schreiber MA**. "Comparison of Inhaled and Intravenous Anesthetic Regimens on Hemodynamics and Inflammation in a Porcine Model of Goal-Directed Resuscitation of Hemorrhagic Shock," 2008. J Surg Res, 144, 425.

13. Cho SD, Holcomb JB, Tieu BH, Englehart MS, Morris MS, Karahan ZA, Underwood SA, Muller PJ, Prince MD, Medina L, Sondeen J, Shults C, Duggan M, Tabbara M, Alam HB, **Schreiber MA**. Reproducibility of An Animal Model Simulating Complex, Combat-Related Injury in a Multiple-Institution Format. Shock. 2009 Jan;31 (1): 87-96.

14. Spoerke N, Zink K, Cho SD, Differding J, Muller P, Karahan A, Sondeen J, Holcomb JB, **Schreiber M**. Lyophilized Plasma for Resuscitation in a Swine Model of Severe Injury. Archives of Surgery. 2009 Sep;144(9):829-834.

15. Alam HB, Bice LM, Butt MU, Cho SD, Dubick MA, Duggan M, Englehart MS, Holcomb JB, Morris MS, Prince MD, **Schreiber MA**, Shults C, Sondeen JL, Tabbara M, Tieu BH, Underwood SA; Hemostatic Resuscitation Research Group. Testing of blood products in a polytrauma model: results of a multi-institutional randomized preclinical trial. Journal of Trauma. 2009 Oct;67(4):856-64.

16. Spoerke NJ, Van PY, Differding JA, Zink KA, Cho SD, Muller PJ, Karahan ZA, Sondeen JL, Holcomb JB, **Schreiber MA**. Red blood cells accelerate the onset of clot formation in polytrauma and hemorrhagic shock. Journal of Trauma. 2010 Nov;69(5):1054-1059; discussion 1059-61.

17. Van PY, Spoerke N, Sambasivan CN, Kremenevskiy I, Hamilton G, Differding JA, Watters JM, **Schreiber MA**. Lyophilized plasma reconstituted with ascorbic acid suppresses inflammation and oxidative DNA damage. J Trauma. 2011 Jul;71(1):20-4 discussion 24-5.

18. Van PY, Holden WE, Spoerke NJ, Sambasivan CN, Differding JA, Watters JM, Phillips CR, **Schreiber MA**. Nitric oxide regulates auto-resuscitation in hemorrhagic shock. Shock. 33(1): 45, 2010.

19. Hamilton GJ, PY Van, NJ Spoerke, I Kremenevskiy, JA Differding, CN Sambasivan, JM Watters, and **MA Schreiber**. Lyophilized Plasma With Ascorbic Acid Decreases Inflammation in Hemorrhagic Shock. J Trauma.2011.Aug;71(2)292-97discussion 297-98.

20. Hamilton GJ, NJ Spoerke, AA Alexandridis, I Kremenevskiy, PY Van, SD Cho, KA Zink, JA Differding, ZA Karahan, JM Watters, JL Sondeen, JB Holcomb, and **MA Schreiber**. Lyophilized Plasma Decreases Pro-Inflammatory Cytokine Synthesis In Severe Hemorrhagic Shock. *Shock*. 2010;33(7) (Suppl 1):14-87.

21. Riha GM, Kunio NR, Van PY, Hamilton GJ, Anderson R, Differding JA, **Schreiber MA**. Hextend and 7.5% hypertonic saline with dextran are equivalent to Lactated Ringer's in a swine model of initial resuscitation of uncontrolled hemorrhagic shock. J Trauma. 2011 Dec;71(6):1755-60.

ABSTRACTS

1. Todd SR, Malinoski D, **Schreiber MA**. Lactated Ringer's is Superior to Normal Saline in Uncontrolled Hemorrhagic Shock. Shock. 2003;Supplement to Volume 19:169.
2. Todd SR, Malinoski D, **Schreiber MA**. Hextend Attenuates the Hypercoagulability Following Severe Liver Injury in Swine. Journal of Trauma. 2004;56:226.
3. Stefater, Brundage SI, Watters J, Zautke N, Todd SR, Lam, **Schreiber MA.** Lacated Ringer's does not Increase Inflammation after Shock. Shock 2004;Supplement to Volume 21:458.
4. Differding JA, Watters JM, Muller PJ, **Schreiber MA**. The Correlation between Mean Arterial Blood Pressure and Tissue Oxygenation after Uncontrolled Hemorrhagic Shock. Journal of Surgical Research. 2004;126:336-337.
5. Watters JM, Jackson T, Muller PJ, Malinoski D, Todd SR, **Schreiber MA**. Fluid Resuscitation Increases Inflammatory Response to Traumatic Injury. Journal of Trauma. 2004;57:1378.
6. Watters JM, Differding JA, Muller PJ, **Schreiber MA**. A Single Bolus of 3% Hypertonic Saline with Dextran Provides Optimal Resuscitation after Uncontrolled Hemorrhagic Shock. Journal of Trauma. 2005;58:216.
7. Wiesberg TT, Differding JA, Enomoto TM, Sawai RS, Muller PJ, **Schreiber MA**. Resuscitation with Lactated Ringer's (LR) vs. Normal Saline (NS) Leads to a Hypercoagulable State in an Uncontrolled Hemorrhagic Shock Swine Model. Journal of Trauma. 2006;59:1538.
8. Englehart M, Tieu B, Kiraly L, Sawai R, **Schreiber MA**. The Influence of Anesthesia on a Swine Model of Hemorrhagic Shock. Shock. 2006;25:58.
9. Tieu B, Enomoto TM, Phillips C, Hagg D, Wiesberg T, Muller P, **Schreiber MA**. Resuscitation with Normal Saline (NS) Increased Volume Requirement, Increased Cardiac Output and Decreased Systemic Vascular Resistance compared to Lactated Ringers (LR) in an Uncontrolled Hemorrhagic Shock Model in Swine. Journal of the American College of Surgeons. 2006;32:S32.
10. Kiraly LN, Sawai RS, Watters JM, Differding JA, Wiesberg TT, Muller PJ, **Schreiber MA**. 7.5% Saline with Dextran Resuscitation Causes Dysfunctional Inflammation in Uncontrolled Hemorrhagic Shock. Journal of Trauma. 2006;61:1559.
11. Englehart MS, Kiraly LN, Tieu BH, Sawai RS, Underwood SA, Muller PJ, **Schreiber MA**. Ketamine-Based Total Intravenous Anesthesia (TIVA) is Superior to Isoflurane (ISO) in a Swine Model of Hemorrhagic Shock. Journal of Trauma. 2006;61:1559.
12. Sondeen J, Shults C, Holcomb J, Alam H, **Schreiber MA**. Reproducibility of a Complex Hemorrhage Shock Model and Tissue Injury in Swine at Three Academic Research Centers. Shock. 2007;27:74.

Presentations

1. Todd SR**,** *Hextend Attenuates Hypercoagulability Following Severe Liver Injury in Swine.* – 17th Annual Meeting of the Eastern Association for the Surgery of Trauma – Amelia Island FL; 2004.

2. Todd SR**,** *Fluid Resuscitation in Uncontrolled Hemorrhagic Shock* – The University of Texas Surgical Grand Rounds – Houston, TX; 2003.

3. Todd SR**,** *Fluid Resuscitation in Uncontrolled Hemorrhagic Shock* – Oregon Health & Science University Surgical Grand Rounds – Portland, OR; 2003.

4. Todd SR**,** *Lactated ringer's is superior* to *Normal Saline in Uncontrolled Hemorrhagic Shock* - Presented at the 26th Annual Conference on Shock; Phoenix AZ - June 2003

5. Watters JM, A Single Bolus of 3.5% Hypertonic Saline with Dextran Provides Optimal Resuscitation after Uncontrolled Hemorrhagic Shock. Western Trauma Association, Jackson Hole, WY, Mar 2005.

6. Watters JM, Fluid Resuscitation Increases Inflammatory Response to Traumatic Injury. Eastern Association for the Surgery of Trauma, Ft. Lauderdale, FL, Jan 2005.

7. Watters JM, Fluid Resuscitation Following Uncontrolled Hemorrhagic Shock. Grand Rounds, Division of Pulmonary and Critical Care Medicine, Oregon Health & Science University, Portland, OR, Jan 2005.

8. Watters JM, A Single Bolus of 3.5% Hypertonic Saline with Dextran Provides Optimal Resuscitation after Uncontrolled Hemorrhagic Shock. Committee on Trauma, Regional Presentation, Olympia, WA, Dec 2004.

9. Watters JM, A Single Bolus of 3.5% Hypertonic Saline with Dextran Provides Optimal Resuscitation after Uncontrolled Hemorrhagic Shock. ATACCC, FL, Aug 2004.

10. Watters JM, Resuscitation with lactated Ringer's does not increase inflammatory response in a swine model of uncontrolled hemorrhagic shock. Society of University Surgeons, St. Louis, MO, Feb 2004.

11. Sawai R, *Increasing resuscitation fluid tonicity does not increase the inflammatory response in uncontrolled hemorrhagic shock*. 66th Annual Meeting of the Society of University Surgeons, Nashville, Tennessee. February 2005.

12. Sawai R, *7.5% Saline with dextran resuscitation causes dysfunctional inflammation in uncontrolled hemorrhagic shock*. American College of Surgeons Committee on Trauma Regional Competition, Region X, Olympia, Washington. December 2004.

13. Kiraly LN, "7.5% Saline with Dextran Resuscitation Causes Dysfunctional Inflammation in Uncontrolled Hemorrhagic Shock." January 17, 2007 Eastern Association of Surgery of Trauma.

14. Kiraly LN, "Resuscitation with Normal Saline vs. Lactated Ringers Modulates Hypercoagulability and Leads to Increased Blood Loss in an Uncontrolled Hemorrhagic Shock Swine Model." June 19, 2006 American College of Surgeons, Oregon & Washington Chapter Meeting.

15. Kiraly LN, "7.5% Saline with Dextran Resuscitation Causes Dysfunctional Inflammation in Uncontrolled Hemorrhagic Shock." June 16, 2006 Portland Surgical Association.

16. Kiraly LN, "Resuscitation with Normal Saline vs. Lactated Ringers Modulates Hypercoagulability and Leads to Increased Blood Loss in an Uncontrolled Hemorrhagic Shock Swine Model." January 11, 2006 Eastern Association of Surgery of Trauma. Alexander Resident Paper Competition.

17. Tieu BH, Fluid Resuscitation Increases Inflammatory Gene Transcription Following Traumatic Injury. The Scientific Session of the 59th Annual Meeting of the Portland Surgical Society 2006.

18. Tieu BH, Resuscitation with Normal Saline Increases Volume Requirement, Increases Cardiac Output and Decreases Systemic Vascular Resistance Compared to Lactated Ringers in an Uncontrolled Hemorrhagic Shock Model in Swine. American College of Surgeons 92nd Annual Clinical Congress Critical Care Session II 2006.

19. Tieu BH, Reproducibility of a Complex Hemorrhagic Shock Model and Tissue Injury in Swine at Three Academic Research Centers. Shock Society: 30th Annual Conference on Shock. June 12th, 2007.

20. Englehart MS, The Influence of Anesthesia on a Swine Model of Hemorrhagic Shock - Region X American College of Surgeons Committee on Trauma Paper Competition; December 2005

21. Englehart MS, Ketamine-Based Total Intravenous Anesthesia is Superior to Isoflurane in a Swine Model of Hemorrhagic Shock - OR/WA Annual Regional State Chapter Meeting of the American College of Surgeons; June 2006

22. Englehart MS, Ketamine-Based Total Intravenous Anesthesia is Superior to Isoflurane in a Swine Model of Hemorrhagic Shock - Portland Surgical Society; June 2006

23. Englehart MS, Ketamine-Based Total Intravenous Anesthesia (TIVA) is Superior to Isoflurane (ISO) in a Swine Model of Hemorrhagic Shock - Eastern Association for the Surgery of Trauma, Raymond H. Alexander M.D. Resident Paper Competition; January 2007

24. Englehart MS, Lyophilized plasma with vitamin C suppresses inflammation in a swine model of severe injury - Region X American College of Surgeons Committee on Trauma Paper Competition; December 2009

25. Gee AC, "Comparison of Inhaled and Intravenous Anesthetic Regimens on Hemodynamics andInflammation in a Porcine Model of Goal-Directed Resuscitation of Hemorrhagic Shock," Oral Presentation and abstract. American College of Surgeons Committee on Trauma, Region X Resident Trauma Paper Competition, Olympia, WA, 2007.

26. Cho SD, Reproducibility of a Complex Hemorrhagic Shock and Tissue Injury Model in Swine at Three Academic Research Centers. Podium presentation at the Region X meeting of the American College of Surgeons Committee on Trauma, Dec 1, 2007.

27. Allison CE, "Comparison of inhaled and intravenous anesthetic regimens on hemodynamics and inflammation in a porcine model of hemorrhagic shock." Academy of Academic Surgeons/Society of University Surgeons, Huntington Beach, CA. 2/2008

28. Spoerke N, The Use of Lyophilized Plasma for Resuscitation in a Swine Model of Severe Injury – ACS Region X Committee on Trauma, 11/15/08

29. Spoerke N, The Use of Lyophilized Plasma for Resuscitation in a Swine Model of Severe Injury – Pacific Coast Surgical Association, 2/15/09, San Francisco, CA

30. Spoerke N, The Presence of RBC Accelerates the Onset of Clot Formation in Polytrauma and Hemorrhagic Shock - Portland Surgical Society, 5/29/09, Portland, OR

31. Spoerke N, Lyophilized Plasma for Resuscitation in a Swine Model of Severe Injury – Annual meeting of the OR/WA chapter of the American College of Surgeons, 6/15/09, Lake Chelan, WA

32. Spoerke N, Lyophilized Plasma for Resuscitation in a Swine Model of Severe Injury – United States Military Health Research Forum, 9/2/09, Kansas City, MO

33. Spoerke N, Red Blood Cells Accelerate the Onset of Clot Formation in Polytrauma and Hemorrhagic Shock – Annual meeting of the Eastern Association for the Surgery of Trauma (EAST), 1/17/2010, Phoenix, AZ

34. Van PY, The presence of red blood cells accelerates the onset of clot formation in polytrauma and hemorrhagic shock. American College of Surgeons, Committee on Trauma, Region X Meeting, November 2009, Olympia, Washington.

35. Van PY, The presence of red blood cells accelerates the onset of clot formation in polytrauma and hemorrhagic shock. American College of Surgeons, Committee on Trauma Resident Competition, March 2010, Las Vegas, Nevada.

36. Van PY, Nitric oxide regulates auto-resuscitation in hemorrhagic shock. Oregon/Washington Chapter American College of Surgeons Meeting, June 2010, Sunriver, Oregon

37. Van PY, Nitric oxide regulates auto-resuscitation in hemorrhagic shock. Shock Society Annual Conference, June 2010, Portland, Oregon.

38. Van PY, The presence of red blood cells accelerates the onset of clot formation in polytrauma and hemorrhagic shock. Oregon/Washington Chapter American College of Surgeons Meeting, June 2010, Sunriver, Oregon

39. Van PY, Lyophilized plasma reconstituted with ascorbic acid suppresses inflammation and oxidative DNA damage. American Association for the Surgery of Trauma Annual Meeting, September 2010, Boston, Massachusetts.

40. Van PY, Lyophilized plasma reconstituted with ascorbic acid suppresses inflammation and oxidative DNA damage. American College of Surgeons, Committee on Trauma, Region X Meeting, November 2010, Tacoma, Washington.

41. Hamilton GJ, Lyophilized Plasma With Ascorbic Acid Decreases Inflammation in Hemorrhagic Shock. Podium Presentation, Annual Scientific Assembly of the Eastern Association for the Surgery of Trauma, Naples, FL. Jan 2011.

42. Hamilton GJ, Lyophilized Plasma Decreases Pro-Inflammatory Cytokine Synthesis In Severe Hemorrhagic Shock. Poster Presentation, Shock Society's 33rd Annual Conference, Portland, OR. Jun 2010. Abstracts. *Shock*. 2010;33(7) (Suppl 1):14-87.

43. Hamilton GJ, Lyophilized Plasma Decreases Pro-Inflammatory Cytokine Synthesis In Severe Hemorrhagic Shock. Podium Presentation, Portland Surgical Society Scientific Session, Portland, OR. May 2010.

44. Riha GM, Hextend and 7.5% Hypertonic Saline with Dextran are Equivalent to Lactated Ringer's in a Swine Model of Initial Resuscitation of Uncontrolled Hemorrhagic Shock. Presented at the 82nd Annual Meeting of the Pacific Coast Surgical Association, Scottsdale, AZ, February 19, 2011.

45. Riha GM, Initial resuscitation in a model of uncontrolled hemorrhage in swine. Presented at the Oregon Chapter of Critical Care Medicine Fall Symposium, Vancouver, Washington, November 15, 2010.

46. Riha GM, Initial resuscitation in a model of uncontrolled hemorrhage in swine. Presented at the Region X, COT, Tacoma Washington, November 13, 2010.

47. Riha GM, Uncontrolled hemorrhagic shock results in hypercoagulable state modulated by initial fluid resuscitation regimens. Presented at the 6th combined annual meeting of the Oregon and Washington state chapters of the American College of Surgeons, Lake Chelan, Washington, June 18, 2011

Awards

1. Watters JM, Winner, Region X Committee on Trauma Basic Science Abstract 2004

2. Watters JM, Winner, Western Trauma Association, Resident Paper Competition 2005

3. Watters JM, Finalist, Eastern Association for the Surgery of Trauma, Raymond H. Alexander Competition 2005

4. Kiraly LN, Winner, Baker-Moseley Award, Clinical Research, Oregon Chapter American College of Surgeons, June 2007

5. Englehart MS, Winner, Best Basic Science Presentation - Region X American College of Surgeons Committee on Trauma Paper Competition 2005

6. Englehart MS, Winner, H. Stephens Mosley M.D. Award for Excellence in Basic Science Research Portland Surgical Society 2006

7. Englehart MS, Winner, Best Manuscript – Eastern Association for the Surgery of Trauma, 2007

8. Englehart MS, Winner, Raymond H. Alexander M.D. Award, Resident Paper Competition, Eastern Association for the Surgery of Trauma, Annual Meeting 2007

9. Englehart MS, Winner, Best Clinical Presentation – Region X American College of Surgeons Committee on Trauma Paper Competition 2007

10. Englehart MS, Winner, Best Basic Science Presentation – Region X American College of Surgeons Committee on Trauma Paper Competition 2009

11. Gee AC, Winner, Best Basic Science Paper, American College of Surgeons Committee on Trauma, Region X Resident Trauma Paper Competition, 2006

12. Cho SD, Runner up, Region X American College of Surgeons Committee on Trauma Paper Competition, 2007

13. Spoerke N, Winner, Region X American College of Surgeons Committee on Trauma, Basic Science Competition 2008

14. Spoerke N, Winner, Pacific Coast Surgical Association Resident Competition, San Francisco, CA, 2009

15. Spoerke N, Winner, Eastern Association for the Surgery of Trauma Resident Competition, Phoenix, AZ, 2010

16. Van PY, Winner, American College of Surgeons, Committee on Trauma, Region X, Basic Science Resident Paper 2009

17. Van PY, Baker-Mosley Award Winner for Basic Science Research, American College of Surgeons Oregon Chapter 2010

18. Van PY, Winner, American College of Surgeons, Committee on Trauma, Region X, Basic Science Resident Paper, 2010

19. Riha GM, Winner Oregon Chapter of the Society of Critical Care Medicine Physician Poster Contest 2010

20. Riha GM, Winner, Pacific Coast Surgical Association 82nd Annual Meeting, Oregon/Hawaii Caucus 2011

21. Anderson R, Winner – Medical Student Oral Research Forum, OHSU May 2011

22. Riha GM, Winner, American College of Surgeons, Committee on Trauma, Region X, Basic Science Resident Paper, 2011

Study Salaried Personnel

Dr. Martin A. Schreiber, MD FACS (Principal Investigator)
Jerome A Differding, MPH (Research Coordinator)
Samantha J Underwood, MS (Research Coordinator)
Patrick Muller, BS (Research Assistant)
Claire Sands, BS (Veterinarian Technician)
Igor Kremenevskiy (Research Assistant / Lab Technician)